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**The pharmacokinetics of lopinavir in HIV-infected adults receiving
rifampicin with adjusted doses of lopinavir/ritonavir tablets.**

The Faculty of Health Sciences, University of Cape Town



Submitted in part fulfillment of the requirements for the degree of Master of
Medicine (MMed) in Clinical Pharmacology

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Candidate: Dr Eric Hermann Decloedt (DCLER1001)

Supervisor: Prof Gary Maartens

I declare that this research report is based on original work performed by me and neither the whole work nor any part of it has been, is being, or is to be submitted for another degree to any other university. This work has not been published prior to registration for the abovementioned degree.

Signed by candidate

Eric Decloedt
February 2012

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This mini-dissertation is one of three examination components towards the MMed degree in Clinical Pharmacology and is presented following the guidelines set out by the University of Cape Town. The dissertation contains the following chapters:

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Chapter 1

The structured literature review

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Abbreviations

APV	Amprenavir
ART	Antiretroviral therapy
ATV/r	Atazanavir/ritonavir
AUC	Area under the plasma concentration time curve
CDC	Centers for Disease Control and Prevention
C _{min}	Minimum concentration in plasma
CYP	Cytochrome P450
DNA	Deoxyribonucleic acid
FDA	Food and Drug Agency
HIV	Human immunodeficiency virus
IDV	Indinavir
IDV/r	Indinavir/ritonavir
INH	Isoniazid
LPV	Lopinavir
LPV/r	Lopinavir/ritonavir
MeSH	Medical subject heading
NRTIs	Nucleoside reverse transcriptase inhibitors
PI	Protease inhibitor
PXR	Pregnane X nuclear receptor
P-gp	P-glycoprotein
RTV	Ritonavir
RXR	Retinoid X receptor
RZ	Rifampicin and pyrazinamide
SQV/r	Saquinavir/ritonavir

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1. Aim of the review

This review focuses on the pharmacokinetics, safety and effectiveness of adjusted-dose protease inhibitors with rifampicin.

2. Introduction

Globally Sub-Saharan Africa carries the biggest burden of patients infected with human immunodeficiency virus (HIV).(1) Tuberculosis is the most common opportunistic infection in patients infected with HIV.(2) Although antiretroviral therapy (ART) has decreased the burden of tuberculosis in HIV-infected patients, the incidence of tuberculosis remains higher than in the general population.(3) HIV-tuberculosis co-infection requires dual treatment with ART and tuberculosis treatment, exposing patients to multiple drug-drug interactions. As ART programs mature, more patients will be changed from first-line to second-line ART. In South Africa, the adult second-line ART consists of the protease inhibitor (PI) lopinavir/ritonavir (LPV/r) and 2 nucleoside reverse transcriptase inhibitors (NRTIs). This review will focus on the data of the drug-drug interactions between the PIs and rifampicin, with an emphasis on LPV/r.

3. Protease inhibitor-rifampicin interaction

Rifampicin is a potent inducer of several drug metabolizing enzymes, particularly of the cytochrome P450 (CYP) 3A4 enzyme.(4) The rifampicin mediated increase in CYP450 enzyme protein synthesis is a complex process that is mediated by the pregnane X nuclear receptor (PXR). Rifampicin binds to PXR forming a rifampicin-PXR complex, which in turns forms a complex with the retinoid X receptor (RXR). The rifampicin-PXR-RXR complex binds to a deoxyribonucleic acid (DNA) upstream promoter element, recruits a co-activator that binds

to the TATA box binding protein (TBP); and ultimately enhances CYP3A4 DNA transcription.(4) The potent induction effect of rifampicin is not confined to CYP450 enzymes, but also increases the expression of transporter proteins, such as p-glycoprotein (p-gp). P-gp is a transmembrane efflux protein that is expressed in the liver, small intestine and other organs that actively transport xenobiotics out of the organ cells.(5) PIs are substrates of both CYP3A4 and p-gp. Rifampicin induction of CYP3A4 and p-gp reduces plasma PI trough concentrations by more than 90%, reducing PI efficacy.(6)

The PI-rifampicin interaction can be avoided by replacing rifampicin with the rifamycin rifabutin, which has a minimal inducing effect on CYP3A4.(6) High-income countries therefore replace rifampicin with rifabutin in patients on PI-based ART who have tuberculosis. However, rifabutin is not currently used in developing countries for several reasons. First, PIs inhibit the metabolism of rifabutin resulting in a marked increase in the area under the plasma concentration-time curve (AUC) of rifabutin and its metabolite 25-O-des-acetyl rifabutin.(6) Rifabutin therefore requires a dose reduction and a decrease in dosing frequency from daily to 3 times a week, which will be difficult to implement in clinics where standard tuberculosis drugs are administered daily. Second, current tuberculosis treatment programs dose standardized tuberculosis treatment regimens in fixed-dosed tablets and rifabutin dosing will require separate dosing of individual anti-tuberculosis drugs. Last, rifabutin is priced out of range for use in most developing countries. Therefore developing countries are currently compelled to use rifampicin-based tuberculosis treatment.

4. Adjusted doses of protease inhibitors in adults when dosed with rifampicin

In order to compensate for the induction of PI metabolism by rifampicin, two dosing strategies have been explored. The dose of the PI can be increased or alternatively the dose of co-administered ritonavir (RTV), used for its “boosting” effect on other PIs, can be increased. RTV is a potent inhibitor of CYP450 enzymes, including CYP3A4, and can produce substantial increases in the plasma concentrations of other PIs that are CYP3A4 substrates.(7)

5. Objectives of literature review

The primary objective of this review is to review the literature of dose adjusted PIs with rifampicin, focusing on the pharmacokinetics, safety and effectiveness of the PI. The secondary objective is to determine the gaps in current knowledge and the contribution the study will make to existing knowledge.

6. Literature review methodology

6.1 Search strategy

The electronic journal database PubMed, Cochrane Database of Systematic Reviews and Google Scholar was searched from 1994 onwards, to capture all studies from 1995 when the PIs were approved by the United States Food and Drug Agency (FDA). We also searched the electronic conference databases of the International Workshop on Clinical Pharmacology of HIV Therapy, Conferences on Retroviruses and Opportunistic Infections and International AIDS Conference. Our search strategy included the following medical subject heading

(MeSH) terms: protease inhibitors, lopinavir, atazanavir, saquinavir, nelfinavir, indinavir, darunavir, amprenavir, tipranavir and rifampin. We included all fields in our search but limited our search to humans older than 18 years. The search command was "HIV Protease Inhibitors"[Mesh] OR "lopinavir"[Mesh] OR "atazanavir"[Supplementary Concept] OR "saquinavir"[Mesh] OR "nelfinavir"[Mesh] OR "indinavir"[Mesh] OR "darunavir"[Supplementary Concept] OR "amprenavir"[Supplementary Concept] OR "fosamprenavir"[Supplementary Concept] OR "tipranavir"[Supplementary Concept] AND "rifampin"[Mesh] AND ("humans"[MeSH Terms] AND "adult"[MeSH Terms]). We also scrutinized the citations of reviewed articles for any references not identified in our search.

6.2 Eligibility criteria

6.2.1 Types of studies

Clinical trials, randomized or non-randomized trials, as well as observational studies were included.

6.2.2 Types of participants

Studies of healthy volunteers or HIV-infected patients were included. HIV-infected patients could be ART-naïve or ART-experienced.

6.2.3 Exposure of interest

The exposure of interest was rifampicin co-administered with a PI. Rifampicin could be part of rifampicin-based tuberculosis therapy or be administered on its own. The PI could be part of an ART-regimen or administered on its own.

6.2.4 Outcome measures

Key plasma pharmacokinetic parameters of the PI administered with and without rifampicin were extracted. The key pharmacokinetic parameters included trough concentrations and AUC. All adverse events that occurred in participants on the rifampicin-PI were reviewed, but the focus was on hepatotoxicity.

6.3 Quality criteria

Sixteen studies met the inclusion criteria. Fourteen studies were prospective clinical trials, of which 8 were performed in healthy volunteers; and 2 were retrospective studies. All the prospective clinical trials were open-label studies and some of the treatment arms were randomized to different dosing approaches. Methods were clearly stated and included the inclusion criteria, drug interventions and pharmacokinetic sampling schedule. All the trials reported that ethical approval was granted before any study procedures were performed. No formal sample size calculation was done in any of the trials and sample sizes varied between 2 and 30 participants.

7. Literature summary

Patient data of adjusted dose PI-based ART regimens with rifampicin-based tuberculosis treatment is scarce. Most studies were conducted in healthy normal volunteers and hepatotoxicity occurred frequently. The different PIs dosed with rifampicin will be discussed next.

7.1 Lopinavir

In South Africa LPV/r is part of the second-line ART regimen. Before the publication of our study, dosing recommendations were based on a healthy volunteer study done in the Netherlands.⁽⁸⁾ The study demonstrated that lopinavir (LPV) trough concentrations similar

to those without rifampicin can be achieved, either by adding RTV to give a LPV: RTV ratio of 1:1, or by doubling the dose of the capsule formulation of LPV/r when dosed with rifampicin. From days 1 to 10 all volunteers received LPV/r dosed at 400/100 mg 12 hourly, followed by the same dose of LPV/r but with rifampicin 600 mg daily added from days 11 to 15. After day 15, volunteers were randomized to rifampicin with either double doses of LPV/r 800/200 mg (arm 1) 12 hourly or additional RTV (LPV/r 400/400 mg) (arm 2). Intensive pharmacokinetic sampling was performed on days 10 and 24. Thirty-two volunteers were included in the study and 19 volunteers completed the pharmacokinetic sampling on day 24. Thirteen volunteers prematurely discontinued the study due to adverse events or laboratory abnormalities. Table 1 summarizes the LPV pharmacokinetic data. Both approaches achieved mean LPV trough concentrations (C_{min}) above the recommended minimum target concentration of 1 mg/L for PI-naïve patients.(9) In the two arms 37% (7/19) of the volunteers developed asymptomatic transaminitis. More volunteers discontinued study treatment in the LPV/r 400/400 mg arm compared to the LPV/r 800/200 mg arm (5/9 compared to 2/10).

Subsequent to this study, a LPV/r tablet formulation with reduced pharmacokinetic variability and higher bioavailability unrelated to meals became available; and rifampicin in combination with adjusted doses of the LPV/r tablet formulation in healthy normal volunteers was studied.(10) The volunteers received 600 mg rifampicin daily on days 1 to 5, followed by LPV/r dosed at 600/150 mg or 800/200 mg 12 hourly on days 6 to 15. Unexpected high rates of hepatotoxicity occurred with 8 of 11 volunteers developing symptomatic grade 3 or 4 transaminitis. The study was prematurely discontinued.

The treatment of co-infected HIV-tuberculosis patients on LPV/r-based ART was described in a small retrospective observational study in the Netherlands.⁽¹¹⁾ The study included 34 patients, 26 of whom started concomitant treatment after the recommendation of LPV/r dose adjustment was in the public domain. Only 5/26 patients were dosed correctly with adjusted dose LPV/r. Plasma LPV trough concentrations were measured in 6 patients receiving unadjusted or incorrectly adjusted LPV/r doses and in 2 patients receiving the recommended adjusted LPV/r dose. The LPV trough concentration was therapeutic in 2/6 patients with unadjusted or incorrectly adjusted LPV/r doses, compared to 2/2 patients with correctly adjusted LPV/r doses. In the patients in whom a viral load was measured, more patients in the unadjusted or incorrectly adjusted LPV/r groups had unsuppressed viral loads compared to the correctly adjusted LPV/r patient group (5/8 compared to 3/3). Within 4 weeks of starting the combination treatment, 7 of the 34 patients prematurely stopped the combination because of unspecified acute adverse effects; of whom 2 were on the correctly adjusted dose of LPV/r.

7.2 Saquinavir

The FDA and Roche Pharmaceuticals contraindicated the combination of saquinavir/ritonavir (SQV/r) with rifampicin because of high rates of hepatotoxicity in healthy normal volunteers.⁽¹²⁻¹⁴⁾ Twenty-eight healthy normal volunteers were randomized to either receive SQV/r 1000/100 mg 12 hourly for 14 days, followed by concomitant rifampicin for 14 days; or in arm two, rifampicin for 14 days followed by concomitant SQV/r 1000/100 mg 12 hourly for 14 days.^(12,15) The study was stopped after 11 volunteers on the SQV/r-rifampicin combination developed hepatotoxicity. The drug initiation sequence was an important predictor of hepatotoxicity, as more hepatotoxicity

occurred in the arm where rifampicin was initiated first (9/9 compared with 2/8). Possible explanations for this will be discussed in more detail in section 7.6 below.

Apart from tolerability concerns, dosing SQV/r to get adequate SQV exposure in the presence of rifampicin was challenging. Unboosted SQV 1200 mg 8 hourly with rifampicin decreased the SQV AUC exposure by 70% and 46% in healthy normal volunteers and HIV-infected patients respectively.(16) Boosted SQV were subsequently studied at different doses and dosing intervals. A retrospective study compared HIV-tuberculosis co-infected patients receiving SQV/r-based ART dosed at 1000/100 mg 12 hourly (n=14) with 18 patients receiving triple NRTIs (n=18).(17) Pharmacokinetic data was not recorded, but based on the observation that the virological response in the triple-NRTI group was inferior to the SQV/r-based ART treated group, the authors suggested that the latter combination may be more effective when dosed with rifampicin-based tuberculosis treatment. Once daily SQV/r 1600/200 mg studied in HIV-infected patients on tuberculosis treatment was inadequate to compensate for the rifampicin induction: SQV AUC and trough concentrations were reduced by 39.5% and 48.7% respectively.(14) A third of patients (6/18) had unsuppressed viral loads after completing tuberculosis treatment, and 1 of 18 patients developed asymptomatic grade 3 hepatitis. Reassuring virological outcomes in HIV-tuberculosis co-infected patients treated with the SQV/r 400/400 mg 12 hourly dosing approach, was reported in 2 small prospective studies (n=2 and n=20 respectively).(18,19) Tolerability was acceptable: no hepatotoxicity occurred in the 2-patient study and 4/20 patients developed symptomatic hepatotoxicity in the other study.(19)

Health care workers from developing countries questioned the FDA's safety recommendation based on limited healthy normal volunteer and published patient

data.(12-14) The health care workers argued that SQV/r- based ART (400/400 mg) has been dosed safely and effectively for years with tuberculosis treatment in their countries. The current US Centers for Disease Control and Prevention (CDC) recommendation is that although the SQV/r-rifampicin combination should preferably be avoided, the recommended SQV/r dose in the presence of rifampicin is 400/400 mg 12 hourly.(6)

7.3 Indinavir

Although indinavir (IDV) initially inhibit the metabolism of rifampicin, the induction effect of rifampicin on IDV is much more pronounced.(20) Low-dose rifampicin (300 mg daily) resulted in an 87% reduction of IDV trough concentrations in HIV-infected patients receiving indinavir/ritonavir (IDV/r)-based ART dosed 800/100 mg 12 hourly.(21) This dramatic reduction in IDV trough concentrations was seen with half the normal dose of rifampicin after only 4 days of treatment, probably underestimating the true effect of rifampicin treatment. This is because rifampicin induction only approaches maximum after at least 10 days of rifampicin administration.(22) No hepatotoxicity occurred after the short duration of rifampicin administration.

7.4 Amprenavir

In healthy normal volunteers standard doses of rifampicin dosed with unboosted amprenavir (APV) 1200 mg 12 hourly, decreased APV AUC exposure by 82% and trough concentrations by 92%.(23) The combination was well tolerated despite rifampicin being dosed before APV. APV production was discontinued and replaced with the pro-drug fosamprenavir that has an improved bioavailability.

7.5 Atazanavir

A healthy normal volunteer study showed that daily dosing of atazanavir/ritonavir (ATV/r) 400/200 mg was inadequate to compensate for the inducing effect of rifampicin.(24) ATV trough concentrations were decreased by 59% when dosed with rifampicin. Increasing the dosing interval of unboosted ATV 300 mg or 400 mg to 12 hourly also failed to maintain adequate plasma exposure in healthy normal volunteers.(25) Once rifampicin was added, ATV trough concentrations decreased by more than 80%. In both studies, steady-state ATV was achieved before rifampicin was introduced. No hepatotoxicity occurred in either cohort. However, when RTV -boosted ATV (300/100 mg) was studied in combination with rifampicin in healthy normal volunteers, the study was discontinued due to hepatotoxicity.(26) The design differed from the previous healthy normal volunteer studies in the sequence of drug initiation: ATV/r was added after rifampicin induced hepatic and gut enzymes for 8 days. One can also not rule out the effect of RTV on the hepatotoxicity as demonstrated in the lopinavir study discussed earlier where more hepatotoxicity occurred in the additional ritonavir arm.(8) Although the study was discontinued before performing plasma ATV sampling, a study of the same dose ATV in 3 HIV-tuberculosis co-infected patients showed dramatic median decreases of 64% and 100% in AUC and trough concentrations respectively.(27)

7.6 Patients different from healthy normal volunteers

From the available PI-rifampicin literature, we know that dose adjusted PIs can compensate for the rifampicin induction effect. However, safety and tolerability concerns deter implementation of this strategy in clinical practice. Unacceptable tolerability was experienced in the healthy normal volunteer studies; but importantly, findings in healthy

normal volunteers may not necessarily be extrapolated to patients co-infected with HIV and tuberculosis.

First, we observed from the healthy normal volunteer studies that volunteers who take the PI before rifampicin, less hepatotoxicity occurred.(8,12,15,25,26) It is speculated that prior induction of the liver enzymes by rifampicin, may give rise to high concentrations of toxic intermediate PI metabolites when the PI is introduced.(26) In high-burden countries rifampicin-based tuberculosis therapy will usually be commenced in patients established on the PI, given that PIs are mostly used in second-line ART regimens. Slower accumulation of metabolites without pre-induction by the rifampicin lead-in may allow tachyphylaxis to ameliorate toxicity.(26) Clinicians may be challenged to differentiate between and deal with tuberculosis- and PI-drug toxicity in the minority of patients who will be started on a PI-based regimen while on tuberculosis treatment.

Second, there may be differences in the risk of hepatotoxicity between healthy normal volunteers and HIV-infected patients. In a multi-national trial that studied the treatment of latent tuberculosis in HIV-infected patients, 12-month isoniazid (INH) was compared with 2-month rifampicin and pyrazinamide (RZ). In HIV-infected patients, allocation to the RZ arm was associated with a hepatotoxicity odds ratio of 4.20 (95% confidence interval of 1.43 to 12.35), while allocation to the RZ arm in healthy normal volunteers was associated with a hepatotoxicity odds ratio of 8.46 (95% confidence interval of 1.9 to 76.5).(28,29) Similar rates of hepatotoxicity occurred in the HIV-infected and HIV-uninfected participants that received INH. The reason for the lower RZ-hepatotoxicity rate observed in HIV-infected patients is not clear, but the hypothesis is that hepatotoxicity is immune-mediated and immunosuppression protects against hepatotoxicity. In addition, active tuberculosis itself

may be immunosuppressive as it has been associated with reduced Th1 lymphocyte counts.(30)

Third, hepatotoxicity may be different in patients receiving combination tuberculosis treatment. Standard tuberculosis treatment consists of multiple drugs, including INH. INH is an inhibitor of CYP3A4 and may therefore theoretically attenuate the induction effect of rifampicin on LPV/r.(31,32)

Lastly, HIV-infected patients with tuberculosis have 2 complex systemic diseases that may influence drug disposition.

Although limited data exists, the use of adjusted dose PIs with rifampicin appears to be better tolerated in HIV-infected patients compared to healthy normal volunteers. See table 2.

7.7 Further research

The proportion of patients on PI-based ART will increase in resource-limited countries as ART programmes mature and more patients move from first-line to second-line ART. Tuberculosis still remains the most common opportunistic infection in HIV-infected patients despite being treated with ART and in the medium-term future the standard of care will be rifampicin-based tuberculosis treatment. Relevant to our setting, the use of LPV/r-based ART in combination with rifampicin needs to be studied in HIV-infected African patients, as the existing evidence is based on small studies in healthy Caucasian male volunteers who are genetically and environmentally different. The LPV/r 400/400 mg dosing approach is complicated by the increased pill burden, complex dosing regimen and low temperature storage instructions. In addition, ritonavir is poorly tolerated in high doses. Furthermore,

there was a trend towards more toxicity in the LPV/r 400/400 mg dosing group compared to the LPV 800/200 mg group.(8) These factors favour the LPV/r 800/200 mg or double dose approach to be evaluated in a bigger study in the relevant patient population.

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8. Tables

Table 1: Steady-state lopinavir pharmacokinetic parameters

LPV/r dosed 12 hourly	AUC ₀₋₁₂ mg·h/liter mean ± SD	Geometric mean ratio (90% CI)	C _{min} mg·h/liter mean ± SD	Geometric mean ratio (90% CI)	C _{max} mg·h/liter mean ± SD	Geometric mean ratio (90% CI)	C ₀ mg·h/liter mean ± SD	Geometric mean ratio (90% CI)
Arm 1 (n=10)								
400/100 mg	111.8 ± 19.0	Referent	6.5 ± 1.8	Referent	12.9 ± 2.5	Referent	7.6 ± 2.4	Referent
800/200 mg + rifampicin	104.5 ± 46.9 p=0.27 ^a	0.84 (0.64-1.10)	5.1 ± 4.2 p=0.08 ^a	0.43 (0.19-0.96)	13.8 ± 4.9 p=0.84 ^a	1.02 (0.85-1.23)	7.0 ± 6.1 p=0.14 ^a	0.46 (0.19-1.10)
Arm 2 (n=9)								
400/100 mg	102.9 ± 26.1	Referent	5.2 ± 1.9	Referent	12.3 ± 3.22	Referent	6.9 ± 2.33	Referent
400/400 mg + rifampicin	100.7 ± 26.8 p=0.81 ^a	0.98 (0.81-1.17)	5.9 ± 2.7 p=0.91 ^a	1.03 (0.68-1.56)	11.5 ± 3.1 p=0.35 ^a	0.93 (0.81-1.07)	7.0 ± 3.5 p=0.64 ^a	0.89 (0.56-1.40)

^aP value for the difference between pharmacokinetic parameters in the two study periods

Table 2: Summary of the hepatotoxicity study discontinuations when protease inhibitors and rifampicin are dosed together

Study	Type of study	Design	Study discontinuations due to hepatotoxicity
Lopinavir			
La Porte <i>et al.</i> (8) (2004)	Healthy normal volunteers	Day 1-10 LPV/r 400/100 mg 12 hourly	
		Day 11-15 LPV/r 400/100 mg 12 hourly & rifampicin	
Nijland <i>et al.</i> (10) (2008)	Healthy normal volunteers	Day 16-24 Arm 1: (n=10) LPV/r 400/400 mg 12 hourly & rifampicin Arm 2: (n=9) LPV/r 800/200 mg 12 hourly & rifampicin	Arm 1: (n=2) Grade II/II transaminits Arm 2: (n=5) Grade II/III transaminits
		Day 1-5 Rifampicin 600 mg daily Day 6-15 Arm 1: (n=5) LPV/r 600/150 mg 12 hourly & rifampicin Arm 2: (n=6) LPV/r 800/200 mg 12 hourly & rifampicin	Arm 1: (n=4) Symptomatic grade IV transaminits Arm 2: (n=4) Symptomatic grade V transaminits Study discontinued
Saquinavir			
Losso <i>et al.</i> (17) (2004)	HIV-tuberculosis co-infected patients	Cohort 1: (n=14) SQV/r-based ART (1000/100 mg) & tuberculosis treatment Cohort 2: (n=18) Triple NRTIs & tuberculosis treatment.	Cohort 1: (n=2) Symptomatic grade III/IV transaminits Cohort 2: (n=3) Symptomatic grade III/V transaminits
Grange <i>et al.</i> (12,15) (2005)	Healthy normal volunteers	Day 1-14 Arm 1: SQV/r 1000/100 mg 12 hourly Arm 2: Rifampicin	
		Day 15-28 Arm 1: (n=8) Arm 2: (n=9) SQV/r 1000/100 mg 12 hourly & rifampicin	Arm 1: (n=2) Arm 2: (n=9) Symptomatic grade II/II transaminits Study discontinued

Table 2 continue: Summary of the hepatotoxicity study discontinuations when protease inhibitors and rifampicin are dosed together

Study	Type of study	Design	Study discontinuations due to hepatotoxicity
Saquinavir continue			
Rolla <i>et al.</i> (19) (2006)	HIV-tuberculosis co-infected patients	Day 1-30 Tuberculosis treatment Day 31-179 Tuberculosis treatment & SQV/r based ART (400/400 mg) 12 hourly (n=20)	Symptomatic grade II/IV transaminitis (n=4)
Ribera <i>et al.</i> (14) (2007)	HIV-tuberculosis co-infected patients	Month 1-2 Tuberculosis treatment Month 3-9 Tuberculosis treatment & SQV/r-based ART (1600/200 mg) 12 hourly (n=18)	Asymptomatic grade III transaminitis (n=1)
Indinavir			
Justesen <i>et al.</i> (21) (2004)	HIV-infected volunteers	Day 1-4 IDV/r-based ART (800/100 mg) 12 hourly & rifampicin (n=6)	No hepatotoxicity
Atazanavir			
Haas <i>et al.</i> (26) (2009)	Healthy normal volunteers	Day 1-8 Rifampicin Day 9-20 ATV/r (300/100 mg) 12 hourly & rifampicin (n=3)	Symptomatic grade II/III/IV transaminitis (n=3) Study discontinued

9. References

- (1) Joint United Nations Programme on HIV/AIDS (UNAIDS) and World Health Organization (WHO) 2009.
AIDS epidemic update : November 2009. 2009; Available at:
http://www.unaids.org/en/media/unaids/contentassets/dataimport/pub/report/2009/jc1700_epi_update_2009_en.pdf. Accessed February 18, 2011.
- (2) Corbett EL, Marston B, Churchyard GJ, De Cock KM. Tuberculosis in sub-Saharan Africa: opportunities, challenges, and change in the era of antiretroviral treatment. *Lancet* 2006 Mar 18;367(9514):926-937.
- (3) Lawn SD, Wood R, De Cock KM, Kranzer K, Lewis JJ, Churchyard GJ. Antiretrovirals and isoniazid preventive therapy in the prevention of HIV-associated tuberculosis in settings with limited health-care resources. *Lancet Infect Dis* 2010 Jul;10(7):489-498.
- (4) Niemi M, Backman JT, Fromm MF, Neuvonen PJ, Kivisto KT. Pharmacokinetic interactions with rifampicin : clinical relevance. *Clin Pharmacokinet* 2003;42(9):819-850.
- (5) Fromm MF. P-glycoprotein: a defense mechanism limiting oral bioavailability and CNS accumulation of drugs. *Int J Clin Pharmacol Ther* 2000 Feb;38(2):69-74.
- (6) CDC. Managing Drug Interactions in the Treatment of HIV-Related Tuberculosis. 2007; Available at: http://www.cdc.gov/tb/publications/guidelines/HIV_AIDS.htm. Accessed 13 August, 2010.

(7) Bartlett JG, Gallant JE, Pham PA. Medical Management of HIV Infection. 2009-2010

Edition ed. Durham, NC: Knowledge Source Solutions, LLC; 2010.

(8) la Porte CJ, Colbers EP, Bertz R, Voncken DS, Wikstrom K, Boeree MJ, et al.

Pharmacokinetics of adjusted-dose lopinavir-ritonavir combined with rifampin in healthy volunteers. *Antimicrob Agents Chemother* 2004 May;48(5):1553-1560.

(9) la Porte CJL, Back DJ, Blaschke T, Boucher CAB, Fletcher CV, Flexner C, et al. Updated guideline to perform therapeutic drug monitoring for antiviral agents. *Rev Antivir Ther* 2006;3:4-14.

(10) Nijland HM, L'homme RF, Rongen GA, van Uden P, van Crevel R, Boeree MJ, et al. High incidence of adverse events in healthy volunteers receiving rifampicin and adjusted doses of lopinavir/ritonavir tablets. *AIDS* 2008 May 11;22(8):931-935.

(11) L'homme RF, Nijland HM, Gras L, Aarnoutse RE, van Crevel R, Boeree M, et al. Clinical experience with the combined use of lopinavir/ritonavir and rifampicin. *AIDS* 2009 Apr 27;23(7):863-865.

(12) Schmitt C, Riek M, Winters K, Schutz M, Grange S. Unexpected Hepatotoxicity of Rifampin and Saquinavir/Ritonavir in Healthy Male Volunteers. *Arch Drug Inf* 2009 Mar;2(1):8-16.

(13) Torres TS, Rolla VC. Saquinavir and rifampicin for tuberculosis and AIDS: new considerations. *Int J Tuberc Lung Dis* 2006 Nov;10(11):1302; author reply 1302-3.

(14) Ribera E, Azuaje C, Lopez RM, Domingo P, Curran A, Feijoo M, et al. Pharmacokinetic interaction between rifampicin and the once-daily combination of saquinavir and low-dose ritonavir in HIV-infected patients with tuberculosis. *J Antimicrob Chemother* 2007 Apr;59(4):690-697.

(15) Unexpected hepatotoxicity observed in a healthy volunteer study on the effects of multiple dose rifampicin on the steady-state pharmacokinetics of ritonavir-boosted saquinavir and vice versa. Sixth International Workshop on Clinical Pharmacology of HIV therapy; 28 -30 April; Montreal, Quebec, Canada; 2005.

(16) Grub S, Bryson H, Goggin T, Ludin E, Jorga K. The interaction of saquinavir (soft gelatin capsule) with ketoconazole, erythromycin and rifampicin: comparison of the effect in healthy volunteers and in HIV-infected patients. *Eur J Clin Pharmacol* 2001 May;57(2):115-121.

(17) Losso MH, Lourtou LD, Toibaro JJ, Saenz C, Gonzalez C. The use of saquinavir/ritonavir 1000/100 mg twice daily in patients with tuberculosis receiving rifampin. *Antivir Ther* 2004 Dec;9(6):1031-1033.

(18) Veldkamp AI, Hoetelmans RM, Beijnen JH, Mulder JW, Meenhorst PL. Ritonavir enables combined therapy with rifampin and saquinavir. *Clin Infect Dis* 1999 Dec;29(6):1586.

(19) Rolla VC, da Silva Vieira MA, Pereira Pinto D, Lourenco MC, de Jesus Cda S, Goncalves Morgado M, et al. Safety, efficacy and pharmacokinetics of ritonavir 400mg/saquinavir 400mg twice daily plus rifampicin combined therapy in HIV patients with tuberculosis. *Clin Drug Investig* 2006;26(8):469-479.

- (20) Jaruratanasirikul S, Sriwiriyan S. Effect of indinavir on the pharmacokinetics of rifampicin in HIV-infected patients. *J Pharm Pharmacol* 2001 Mar;53(3):409-412.
- (21) Justesen US, Andersen AB, Klitgaard NA, Brosen K, Gerstoft J, Pedersen C. Pharmacokinetic interaction between rifampin and the combination of indinavir and low-dose ritonavir in HIV-infected patients. *Clin Infect Dis* 2004 Feb 1;38(3):426-429.
- (22) Reitman ML, Chu X, Cai X, Yabut J, Venkatasubramanian R, Zajic S, et al. Rifampin's acute inhibitory and chronic inductive drug interactions: experimental and model-based approaches to drug-drug interaction trial design. *Clin Pharmacol Ther* 2011 Feb;89(2):234-242.
- (23) Polk RE, Brophy DF, Israel DS, Patron R, Sadler BM, Chittick GE, et al. Pharmacokinetic Interaction between amprenavir and rifabutin or rifampin in healthy males. *Antimicrob Agents Chemother* 2001 Feb;45(2):502-508.
- (24) Burger DM, Agarwala S, Child M, Been-Tiktak A, Wang Y, Bertz R. Effect of rifampin on steady-state pharmacokinetics of atazanavir with ritonavir in healthy volunteers. *Antimicrob Agents Chemother* 2006 Oct;50(10):3336-3342.
- (25) Acosta EP, Kendall MA, Gerber JG, Alston-Smith B, Koletar SL, Zolopa AR, et al. Effect of concomitantly administered rifampin on the pharmacokinetics and safety of atazanavir administered twice daily. *Antimicrob Agents Chemother* 2007 Sep;51(9):3104-3110.
- (26) Haas DW, Koletar SL, Laughlin L, Kendall MA, Suckow C, Gerber JG, et al. Hepatotoxicity and gastrointestinal intolerance when healthy volunteers taking rifampin add twice-daily atazanavir and ritonavir. *J Acquir Immune Defic Syndr* 2009 Mar 1;50(3):290-293.

(27) Mallolas J, Sarasa M, Nomdedeu M, Soriano A, Lopez-Pua Y, Blanco JL, et al.

Pharmacokinetic interaction between rifampicin and ritonavir-boosted atazanavir in HIV-infected patients. *HIV Med* 2007 Mar;8(2):131-134.

(28) Tortajada C, Martinez-Lacasa J, Sanchez F, Jimenez-Fuentes A, De Souza ML, Garcia JF, et al. Is the combination of pyrazinamide plus rifampicin safe for treating latent tuberculosis infection in persons not infected by the human immunodeficiency virus? *Int J Tuberc Lung Dis* 2005 Mar;9(3):276-281.

(29) Jasmer RM, Saukkonen JJ, Blumberg HM, Daley CL, Bernardo J, Vittinghoff E, et al. Short-course rifampin and pyrazinamide compared with isoniazid for latent tuberculosis infection: a multicenter clinical trial. *Ann Intern Med* 2002 Oct 15;137(8):640-647.

(30) Lienhardt C, Azzurri A, Amedei A, Fielding K, Sillah J, Sow OY, et al. Active tuberculosis in Africa is associated with reduced Th1 and increased Th2 activity in vivo. *Eur J Immunol* 2002 Jun;32(6):1605-1613.

(31) Desta Z, Soukhova NV, Flockhart DA. Inhibition of cytochrome P450 (CYP450) isoforms by isoniazid: potent inhibition of CYP2C19 and CYP3A. *Antimicrob Agents Chemother* 2001 Feb;45(2):382-392.

(32) Nishimura Y, Kurata N, Sakurai E, Yasuhara H. Inhibitory effect of antituberculosis drugs on human cytochrome P450-mediated activities. *J Pharmacol Sci* 2004 Nov;96(3):293-300.

Chapter 2

The manuscript

University of Cape Town

Pharmacokinetics of Lopinavir in HIV-Infected Adults Receiving Rifampin with Adjusted Doses of Lopinavir-Ritonavir Tablets[▽]

Eric H. Decloedt,¹ Helen McIlleron,^{1*} Peter Smith,¹ Concepta Merry,²
Catherine Orrell,³ and Gary Maartens¹

Division of Clinical Pharmacology, Department of Medicine, Groote Schuur Hospital, University of Cape Town, Cape Town, South Africa¹; Department of Pharmacology, St. James's Hospital, Trinity College Dublin, Dublin, Ireland²; and Desmond Tutu HIV Foundation, Institute of Infectious Diseases and Molecular Medicine, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa³

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Rifampin coadministration dramatically reduces plasma lopinavir (LPV) concentrations. In healthy volunteers, doubling the dose of a lopinavir-ritonavir (LPV/r) capsule formulation overcame this interaction, but a subsequent study of double doses of the tablet formulation was stopped early owing to hepatotoxicity. However, healthy-volunteer study findings may not apply to HIV-infected adults. We evaluated the steady-state pharmacokinetics of LPV in HIV-infected adults virologically suppressed on an LPV/r regimen who were given rifampin, and the dose of the LPV/r tablet formulation was gradually increased. The steady-state pharmacokinetics of LPV/r were evaluated at baseline, a week after commencing rifampin, a week after the LPV/r dose was increased 1.5 times, and a week after the LPV/r dose was doubled. Twenty-one participants were enrolled. The median [interquartile range (IQR)] predose LPV concentrations (C_0) were 8.1 (6.2 to 9.8) mg/liter at baseline, 1.7 (0.3 to 3.0) mg/liter after 7 days of rifampin, 5.9 (2.1 to 9.9) mg/liter with 1.5 times the dose of LPV/r, and 10.8 (7.0 to 13.1) mg/liter with double-dose LPV/r. There were no significant differences in the LPV area under the plasma concentration-time curve from 0 to 12 h (AUC_{0-12}), C_0 , C_{12} , maximum concentration of drug in serum (C_{max}), or half-life ($t_{1/2}$) between the baseline and double-dose LPV/r time points. Treatment was generally well tolerated, with two participants developing asymptomatic grade 3/4 transaminitis. Doubling the dose of the tablet formulation of LPV/r overcomes induction by rifampin. Less hepatotoxicity occurred in our cohort of HIV-infected participants than was reported in healthy-volunteer studies.

Rifampin is a key component of tuberculosis treatment but also a potent inducer of many cytochrome P450 enzymes and the efflux pump *p*-glycoprotein (15). Protease inhibitors are substrates of both CYP 3A4 and *p*-glycoprotein, and the trough concentrations of all ritonavir-boosted protease inhibitors are reduced by more than 90% when standard doses are coadministered with rifampin (2). A healthy-volunteer study demonstrated that similar lopinavir (LPV) trough concentrations can be achieved either by adding ritonavir (RTV) to give a lopinavir/ritonavir ratio of 1:1 or by doubling the dose of the capsule formulation of lopinavir-ritonavir (LPV/r) (12).

Subsequent healthy-volunteer studies of the interaction between rifampin and adjusted doses of ritonavir-boosted saquinavir, atazanavir, and lopinavir (tablet formulation) were prematurely terminated because of high incidences of hepatotoxicity (6, 7, 16). These high rates of hepatotoxicity in healthy volunteers might not apply to patients with tuberculosis and HIV. First, in the healthy-volunteer studies, initiating rifampin prior to the protease inhibitor was associated with high rates of hepatotoxicity (6, 7, 16). In high-burden countries, protease inhibitors are used as part of the second-line antiretroviral treatment (ART) regimen; hence, most patients

are established on the protease inhibitor before rifampin is initiated. Second, HIV infection may lower the risk of hepatotoxicity, as illustrated by the experience with rifampin and pyrazinamide for latent tuberculosis, which was well tolerated in HIV-infected individuals but was associated with high rates of hepatotoxicity in non-HIV-infected individuals (5, 9, 20).

We evaluated the steady-state pharmacokinetics of LPV and RTV in HIV-infected adults virologically suppressed on an LPV/r regimen who were given rifampin with the dose of LPV/r gradually increased to double the standard dose.

MATERIALS AND METHODS

Study design. The study was an open-label, sequential, four-period, multiple-dose trial in HIV-infected adults who were virologically suppressed (viral loads, <400 copies/ml) on LPV/r together with dual nucleoside reverse transcriptase inhibitors. We compared the steady-state pharmacokinetics of LPV and RTV using noncompartmental analysis under 4 sequential treatment conditions over a 12-h dosing interval in HIV-infected participants: a standard dose of LPV/r (400 mg/100 mg) every 12 hours (study day 1), after which rifampin at 600 mg daily was commenced; LPV/r in standard doses every 12 hours with rifampin (study day 8); 1.5 times the standard dose of LPV/r (600 mg/150 mg) every 12 hours with rifampin (study day 15); and twice the standard dose of LPV/r (800 mg/200 mg) every 12 hours with rifampin (study day 22). Dual nucleoside reverse transcriptase inhibitors were continued throughout with no dose adjustments. Treatment adherence was assessed by using a treatment diary and pill counts.

Study participants. We recruited HIV-infected participants established on an LPV/r regimen (tablet formulation) from a South African antiretroviral clinic, the Hannan Crusaid Treatment Centre in Gugulethu, Cape Town, South Africa. We included medically stable HIV-infected adults older than 18 years with viral loads of <400 copies/ml. Exclusion criteria were abnormal creatinine, severe diarrhea, hepatic disease (defined as either alanine aminotransferase [ALT] at more than 1.5 times the upper limit of normal or a positive hepatitis B surface

* Corresponding author. Mailing address: Division of Clinical Pharmacology, University of Cape Town Medical School, K45, Old Main Building, Groote Schuur Hospital, Observatory 7925, Republic of South Africa. Phone: 27 21 406 6292. Fax: 27 21 448 1989. E-mail: helen.mcilleron@uct.ac.za.

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antigen or hepatitis C antibody test result), grade 3 or higher raised fasting cholesterol (>7.77 mmol/liter) or triglycerides (>8.49 mmol/liter), random glucose measurements of >11.1 mmol/liter, excessive alcohol consumption (in excess of 2 units per day or 14 units per week), symptoms or signs of tuberculosis, taking drugs other than the study drugs known to alter the pharmacokinetics of LPV, and pregnancy.

Pharmacokinetic assessment. Participants were admitted overnight and fasted from 22h00. We observed the dose of LPV/r taken the evening before pharmacokinetic sampling and ensured it was 12 h before the predose sample the next morning. Intensive pharmacokinetic sampling was done predose and at 1.5 h, 2 h, 2.5 h, 3 h, 4 h, 5 h, 6 h, 8 h, and 12 h after observed dosing. Standardized meals were given between the 2-h and 2.5-h, 5-h and 6-h, and 8-h and 12-h sampling times.

We collected 4-ml blood samples in lithium heparin tubes that were kept on melting ice prior to separation. Within 1 h of sampling, the blood samples were centrifuged at 3,000 rpm for 10 min. Each plasma sample was aliquoted and stored at -80°C until the drug concentration was determined.

Safety monitoring. We monitored ALT and total serum bilirubin 3 times a week from the day before rifampin was started until the end of the study period (study days 1, 4, 6, 8, 11, 13, 15, 18, 20, 22, 25, and 28). Electrocardiograms were done at baseline and repeated at days 15 and 22 after the LPV/r dose increases. All adverse events were recorded and graded according to the grading system of the Division of AIDS (4). Subjects were withdrawn from the study when they developed grade 3 or greater adverse events thought to be related to the study drugs.

Drug assays. We used validated liquid chromatography tandem mass spectrometry (LC/MS-MS) to determine the LPV and RTV concentrations in the plasma samples. Lopinavir and ritonavir were assayed as previously described (17). The assay range for lopinavir was 0.05 to 20 $\mu\text{g/ml}$, and for ritonavir it was 0.025 to 5 $\mu\text{g/ml}$. Inter- and intraday coefficients of variation were below 10% for both drugs. The laboratory participates in the International Interlaboratory Control Program of Stichting Kwaliteitsbewaking Klinische Geneesmiddelenanalyse en Toxicologie (KKGIT) (Hague, Netherlands). LPV and RTV concentrations reported as below the limit of quantification (BLQ) were analyzed as the BLQ concentration divided by 2.

Ethical approval. The study was approved by the University of Cape Town Human Research Ethics Committee. Each volunteer was informed of the objectives, nature, and potential risks of the study. Written informed consent was obtained from every participant.

Statistical analyses. Stata version 11.0 (Stata Corporation, College Station, TX) was used to characterize the pharmacokinetic parameters of LPV and RTV using noncompartmental analyses. The area under the plasma concentration-time curve from 0 to 12 h (AUC_{0-12}) was calculated from a 12-h dosing interval using the linear trapezoidal rule. The predose (C_0) and 12-h (C_{12}) LPV concentrations were determined directly from the concentration-time data.

Normally distributed numerical data were described using means and standard deviations, and the *t* test for paired samples was used for hypothesis testing. The Fisher exact test was used for categorical data hypothesis testing. Numerical data that followed a nonnormal distribution were described using the median and interquartile range (IQR), and the Wilcoxon signed-rank test was used for hypothesis testing. Geometric mean ratios (90% confidence intervals [CIs]) were calculated to compare the AUC_{0-12} and the maximum concentration of drug in serum (C_{max}) on study days 8, 15, and 22 to those on study day 1.

RESULTS

We enrolled 21 black African participants in the study, 18 of whom were female. The mean age \pm standard deviation (SD) was 36.1 ± 7.1 years, the mean body mass index \pm SD was 26.2 ± 5.8 kg/m^2 , and the median (IQR) CD4^+ cell count was 564 (408 to 669) cells/ mm^3 .

Figure 1 shows the median steady-state LPV concentrations over time measured on study days 1, 8, 15, and 22, and the pharmacokinetic parameters are summarized in Table 1.

LPV trough (C_0) concentrations below the recommended lower limit for ART-naïve patients (1 mg/liter) (1, 11) occurred in 0/21 study subjects on day 1, 10/21 ($P < 0.01$) on day 8, 2/20 ($P = 0.23$) on day 15, and 0/18 on day 22. The proportion of participants with subtherapeutic LPV C_{12} concentrations was

higher on all study days than the number of those with subtherapeutic LPV C_0 concentrations: 2/21 on day 1, 18/21 ($P < 0.01$) on day 8, 10/20 ($P < 0.01$) on day 15, and 4/18 ($P = 0.39$) on day 22.

Table 2 summarizes the steady-state ritonavir pharmacokinetic parameters.

Nineteen of the 21 participants completed the study. Two participants were withdrawn from the study owing to grade 3/4 asymptomatic transaminitis; one developed grade 3 transaminitis on the standard dose of LPV/r and rifampin, the other on 1.5 times the standard dose of LPV/r and rifampin. In both participants, the transaminitis resolved after LPV/r and rifampin were withdrawn. Another participant withdrew consent after developing grade 2 nausea on 1.5 times the standard dose of LPV/r and rifampin. Other adverse events were mild but frequent: 6 participants developed grade 1/2 transaminitis, 2 grade 1 hyperbilirubinaemia, 8 grade 1/2 nausea, 2 grade 1/2 diarrhea, and 1 PR interval prolongation (0.198 to 2.14 ms) on double the standard dose of LPV/r. All adverse events resolved. On routine viral-load measurement, all but 2 participants remained virologically suppressed. Adherence was measured at least 3 times a week during the study period by using participant questioning and correlating pill counts with recorded doses in the treatment diary. All participants had 100% adherence during the study period using these measures. Subsequent to the pharmacokinetic study, 2 participants had detectable viral loads measured at their routine clinic visits, which were ascribed to poor adherence.

DISCUSSION

This is the first study of the steady-state pharmacokinetics of adjusted doses of LPV/r with rifampin in HIV-infected adults. Therapeutic LPV C_0 trough concentrations were achieved in all participants by doubling the dose of the tablet formulation of LPV/r, although 18/20 participants achieved therapeutic LPV C_0 trough concentrations with 1.5 times the dose of LPV/r (600 mg/150 mg every 12 hours). In our cohort, we consistently found a higher proportion of participants with subtherapeutic C_{12} trough concentrations than with subtherapeutic C_0 trough concentrations. Subtherapeutic LPV C_{12} trough concentrations were noted on all study days. The combination of LPV/r and rifampin was relatively well tolerated in our cohort of HIV-infected individuals compared with previous healthy-volunteer studies, but this cannot be extrapolated to treating patients with tuberculosis, as we have safety data for only 22 days.

LPV is a substrate of both CYP 3A4 and *p*-glycoprotein, which are inhibited by RTV (22). The increased dose of RTV partially offsets the induction effect of rifampin and, together with the increased dose of LPV, sufficiently overcomes induction by rifampin. However, we report pharmacokinetic measurements only; the effect of the increased dose of LPV/r with rifampin on the virological response is unknown.

Most patients achieved therapeutic LPV C_0 trough concentrations with 1.5 times the dose of LPV/r (600 mg/150 mg every 12 hours), making a dose-down strategy with therapeutic drug monitoring of LPV an option in patients who do not tolerate double-dose LPV/r (800 mg/150 mg every 12 hours).

For patients on protease inhibitors who have no other anti-

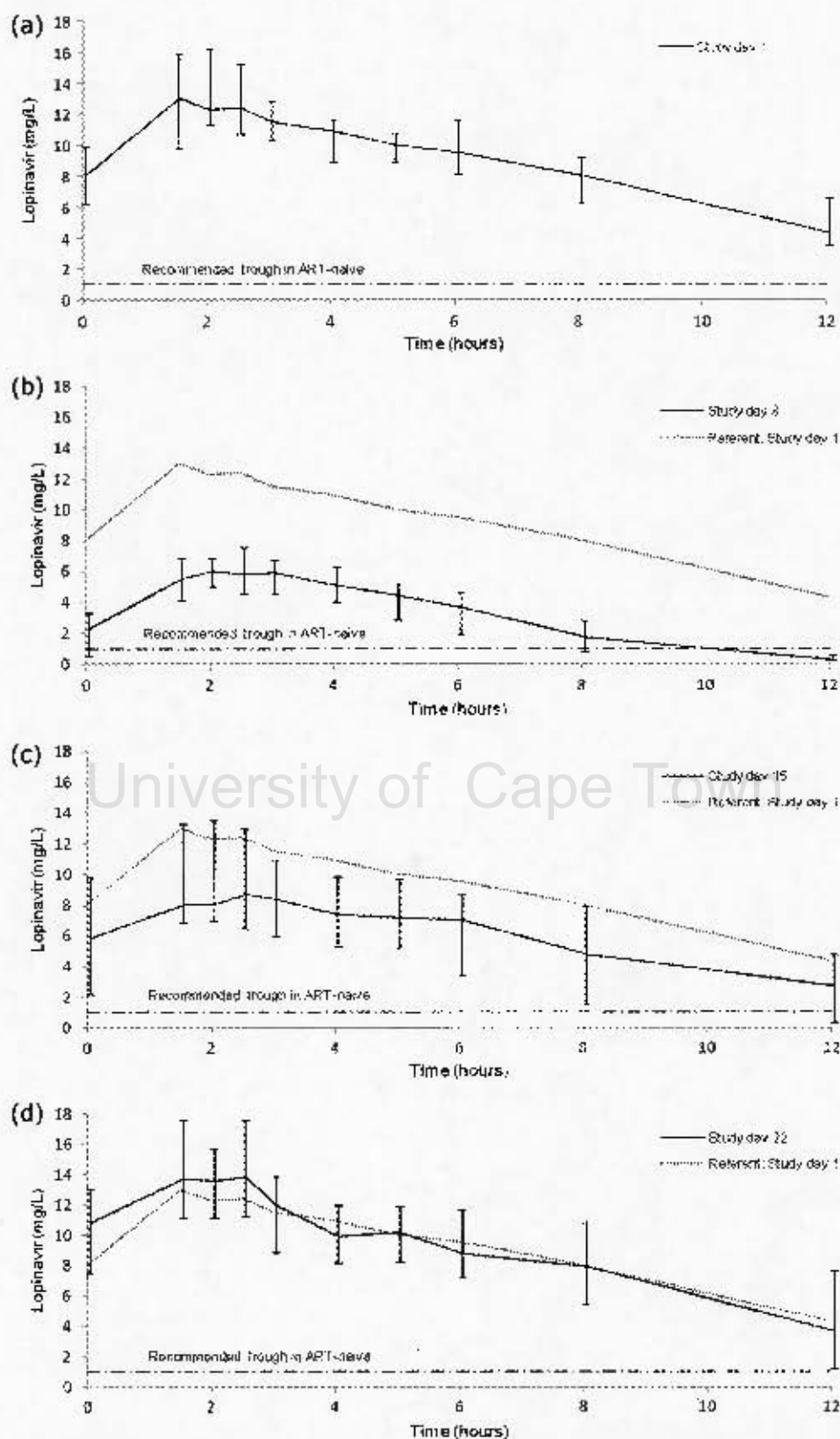


FIG. 1. Median steady-state lopinavir concentrations over time on study days 1 (a), 8 (b), 15 (c), and 22 (d). Shown are median and interquartile lopinavir concentrations over time under the following conditions: study day 1, standard dose of LPV/r (400 mg/100 mg every 12 hours) without rifampin (a); study day 8, standard dose of LPV/r and rifampin (600 mg daily) (b); study day 15, 1.5 times the standard dose of LPV/r and rifampin (c); and study day 22, twice the standard dose of LPV/r and rifampin (d).

TABLE 1. Steady-state lopinavir pharmacokinetic parameters

Study day	AUC ₀₋₂₄ (mg · h/liter) [median (IQR)]	C ₀ (mg/liter) ratio (95% CI) ^a	C _{max} (mg/liter) [median (IQR)]	Geometric mean ratio (95% CI)	t _{1/2} (h) [median (IQR)]	C ₀ (mg/liter) [median (IQR)]	C ₁₂ (mg/liter) [median (IQR)]
1	118.3 (91.3–126.1)	Referent	15.1 (11.3–18.2)	Referent	5.7 (4.6–6.7)	8.1 (6.2–9.8)	4.33 (3.5–6.5)
8	38.2 (25.9–47.0) <i>P</i> < 0.001 ^{a,b}	0.32 (0.28–0.36)	6.3 (5.5–7.9) <i>P</i> < 0.001 ^{a,b}	0.46 (0.42–0.50)	1.6 (1.3–1.8) <i>P</i> < 0.001 ^{a,b}	1.7 (0.3–3.0)	0.2 (0.1–0.4)
15	69.2 (37.4–107.8) <i>P</i> < 0.001 ^{a,b}	0.63 (0.54–0.73)	9.4 (7.0–14.4) <i>P</i> < 0.001 ^{a,b}	0.72 (0.63–0.83)	2.9 (1.3–6.8) <i>P</i> < 0.01 ^{a,b}	5.9 (2.1–9.9)	1.6 (0.1–4.4)
22	101.6 (78.9–141.7) <i>P</i> 0.45 ^{a,b}	1.02 (0.90–1.15)	15.3 (12.2–20.2) <i>P</i> 0.06 ^{a,b}	1.13 (0.97–1.31)	5.0 (2.1–8.7) <i>P</i> 0.5 ^{a,b}	10.8 (7.0–13.1)	3.7 (1.2–7.7)

^a Compared with study day 1.^b Wilcoxon signed-rank test.

retroviral options, two strategies can be followed when treating tuberculosis: adjusting the doses of the protease inhibitor or replacing rifampin with rifabutin. In high-burden countries, rifabutin is seldom an option, owing to its high current cost and complex dosing schedule and the widespread use of fixed-dose combinations containing rifampin for treating tuberculosis. The safety and pharmacokinetics of adjusted-dose LPV/r in combination with rifampin has been studied in two healthy-volunteer studies. La Porte et al. studied the LPV/r capsule formulation and demonstrated that the induction of rifampin can be overcome by either doubling the dose of LPV/r or increasing the RTV component to the same dose as LPV (12). Two of 10 volunteers in the LPV/r 800-mg/200-mg arm and 5 of 9 volunteers in the LPV/r 400-mg/400-mg arm were prematurely discontinued owing to hepatotoxicity. A subsequent study by Nijland et al. evaluating the pharmacokinetics of an adjusted-dose LPV/r tablet formulation with rifampin was prematurely terminated owing to very high rates of hepatotoxicity (16).

There are several possible reasons why lower rates of hepatotoxicity were seen in our study. First, HIV infection may be associated with a lower risk of hepatotoxicity. High rates of hepatotoxicity occurred in non-HIV-infected individuals compared with HIV-infected individuals in studies using rifampin and pyrazinamide to treat latent tuberculosis (5, 9, 20). HIV-tuberculosis-coinfected patients tolerated the combination of rifampin and saquinavir-ritonavir relatively well (14, 18), but high rates of hepatotoxicity were seen in healthy volunteers treated with this combination (6). The lower risk of hepatotoxicity in HIV-infected patients might be explained by an attenuated immune response, which is thought to play an important role in idiosyncratic drug-induced hepatocellular reactions (13). Secondly, we slowly escalated the dose of LPV/r over 2 weeks. High rates of hepatotoxicity occurred in healthy volunteers when double doses of lopinavir-ritonavir were given without dose escalations in combination with rifampin (16). Lastly, we initiated rifampin in HIV-infected participants established on LPV/r. High rates of hepatotoxicity occurred in the healthy-volunteer studies where rifampin was introduced prior to the protease inhibitors (6, 7, 16). Rifampin preinduction may rapidly generate protease inhibitor metabolites that are hepatotoxic (7). In high-burden countries, rifampin-based antitubercular therapy will usually be commenced in patients already on protease inhibitors, given that protease inhibitors are used in second-line ART regimens.

Diurnal variation of protease inhibitors has been reported previously (8, 10). Absorption differences due to the effect of food may account for the differences in our C₀ and C₁₂ trough concentrations. Our cohort received a meal before the observed C₀ dose was taken, while the observed C₁₂ dose was taken after a 10-h fast.

Our study findings have several limitations. First, we assessed the effect of rifampin on LPV/r concentrations only. Tuberculosis is treated with combination antituberculosis drugs, including isoniazid, which is an inhibitor of CYP 3A4 (3). Both LPV/r pharmacokinetics and hepatotoxicity may be different when administered with rifampin and isoniazid. Second, there may also be a disease effect of tuberculosis on both LPV/r concentrations and hepatotoxicity. Third, we evaluated hepatotoxicity for only 3 weeks. It is possible that high rates of

hepatotoxicity may occur later during treatment. There may also be a carryover effect on toxicity owing to the sequential study design, with the last treatment period most affected. Fourth, RTV exposure is known to be higher in females, and our cohort was predominantly female. A greater pharmacokinetic success rate may therefore be seen in female patients (19, 21). Finally, our cohort consisted of HIV-infected participants who were virologically suppressed with high CD4⁺ counts. The risk of hepatotoxicity in this cohort may differ from that in HIV-infected individuals with tuberculosis and various degrees of immunosuppression.

In conclusion, we have described the first evaluation of steady-state pharmacokinetics of adjusted-dose LPV/r and rifampin in HIV-infected adults. We showed that it is possible to overcome the induction effect of rifampin by doubling the dose of LPV/r to 800 mg/200 mg. Compared with previous healthy-volunteer studies, our cohort of HIV-infected adults tolerated the combination of LPV/r and rifampin relatively well. Future research should study the tolerability and effectiveness of double-dose LPV/r in HIV-infected patients with tuberculosis.

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REFERENCES

1. Ananwraich, J., et al. 2005. Pharmacokinetics and 24-week efficacy/safety of dual boosted saquinavir/lopinavir/ritonavir in nucleoside-pretreated children. *Pediatr. Infect. Dis. J.* 24:874–879.
2. Centers for Disease Control and Prevention. 2007. Managing drug interactions in the treatment of HIV-related tuberculosis. http://www.cdc.gov/hiv/publications/guidelines/HIV_AIDS.htm. Accessed 13 August 2010.
3. Ileshi, Z., N. V. Soukhova, and D. A. Mockhart. 2003. Inhibition of cytochrome P450 (CYP450) isoforms by isavudefir, potent inhibition of CYP2C19 and CYP5A. *Antimicrob. Agents Chemother.* 46:392–397.
4. Division of AIDS. National Institute of Allergy and Infectious Diseases, National Institutes of Health. 2004. Division of AIDS table for grading the severity of adult and pediatric adverse events. http://www.niaid.nih.gov/clinicaltrials/boconotes/DAIDS_AE_GradingTable_FinalDec2004.pdf. Accessed 13 August 2010.
5. Gordon, F. M., et al. 2004. Hepatotoxicity of rifampin and pyrazinamide in the treatment of latent tuberculosis infection in HIV-infected persons: is it different than in HIV-uninfected persons? *Clin. Infect. Dis.* 39:561–565.
6. Grange, S., M. Schmitz, C. Schmitt, M. Rick, and E. Gaudeluc-Frühart. 2005. Unexpected hepatotoxicity observed in a healthy volunteer study on the effects of multiple dose rifampicin on the steady-state pharmacokinetics of ritonavir-boosted saquinavir and vice versa, abstract 35, Sixth Int. Workshop Clin. Pharmacol. HIV Ther., Montreal, Quebec, Canada, 28 to 30 April 2005.
7. Haas, D. W., et al. 2009. Hepatotoxicity and gastrointestinal intolerance when healthy volunteers taking rifampin add twice-daily atazanavir and ritonavir. *J. Acquir. Immune Defic. Syndr.* 50:290–293.
8. Hsu, A., et al. 1997. Multiple-dose pharmacokinetics of ritonavir in human immunodeficiency virus-infected subjects. *Antimicrob. Agents Chemother.* 41:898–905.
9. Janssen, R. M., et al. 2002. Short-course rifampin and pyrazinamide compared with isoniazid for latent tuberculosis infection: a multicenter clinical trial. *Ann. Intern. Med.* 137:640–647.
10. Jusken, L. S., and C. Pedersen. 2002. Dermal irritation of plasma protease inhibitor concentrations. *AIDS* 16:2487–2489.
11. La Porte, C. J., et al. 2006. Updated guideline to perform therapeutic drug monitoring for antitubercular agents. *Rev. Antimicrob. Ther.* 3:4–14.
12. La Porte, C. J., et al. 2004. Pharmacokinetics of adjusted-dose lopinavir-ritonavir combined with rifampin in healthy volunteers. *Antimicrob. Agents Chemother.* 48:1553–1560.
13. Lee, W. M. 2003. Drug-induced hepatotoxicity. *N. Engl. J. Med.* 349:474–485.

TABLE 2. Steady-state ritonavir pharmacokinetic parameters

Study day	AUC ₀₋₁₂ (mg · h/liter) [median (IQR)]	Geometric mean ratio (90% CI)	C _{max} (mg/liter) [median (IQR)]	Geometric mean ratio (90% CI)	t _{1/2} (h) (mean ± SD)	C _{tr} (mg/liter) [median (IQR)]	C ₁₂ (mg/liter) [median (IQR)]
1	5.8 (3.9–8.5)	Referent	1.2 (0.8–1.9)	Referent	3.5 (3.2–4.6)	0.33 (0.18–0.55)	0.13 (0.10–0.18)
8	2.2 (1.5–3.7) <i>P</i> < 0.001 ^{a,b}	0.39 (0.34–0.42)	0.5 (0.4–0.9) <i>P</i> < 0.001 ^{a,b}	0.44 (0.38–0.51)	2.6 (2.2–2.8) <i>P</i> < 0.001 ^{a,b}	0.09 (0.03–0.16)	0.03 (0.01–0.05)
15	4.8 (2.5–9.6) <i>P</i> = 0.63 ^{a,b}	0.85 (0.71–1.01)	1.1 (0.7–2.2) <i>P</i> = 0.60 ^{a,b}	0.90 (0.73–1.11)	2.7 (1.9–3.2) <i>P</i> < 0.001 ^{a,b}	0.31 (0.10–0.70)	0.06 (0.03–0.15)
22	7.6 (4.2–13.2) <i>P</i> = 0.004 ^{a,b}	1.42 (1.20–1.67)	2.0 (1.4–3.1) <i>P</i> = 0.002 ^{a,b}	1.54 (1.27–1.88)	2.8 (2.4–3.3) <i>P</i> < 0.003 ^{a,b}	0.63 (0.37–1.11)	0.11 (0.05–0.25)

^a Compared with study day 1.

^b Wilcoxon signed-rank test.

14. Losso, M. H., L. D. Lourtiau, J. J. Tolbaro, C. Saenz, and C. Gonzalez. 2004. The use of saquinavir/zidovudine 1000/100 mg twice daily in patients with tuberculosis receiving rifampin. *Antivir. Ther.* 9:1031–1033.
15. Niemelä, M., J. T. Backman, M. F. Fromm, P. J. Neuvonen, and K. T. Kivisto. 2003. Pharmacokinetic interactions with rifampicin: clinical relevance. *Clin. Pharmacokinet.* 42:819–850.
16. Nijland, H. M., et al. 2008. High incidence of adverse events in healthy volunteers receiving rifampicin and adjusted doses of lopinavir/ritonavir tablets. *AIDS* 22:931–935.
17. Ren, Y., et al. 2008. Effect of rifampicin on lopinavir pharmacokinetics in HIV-infected children with tuberculosis. *J. Acquir. Immune Defic. Syndr.* 47:568–569.
18. Ribera, E., et al. 2007. Pharmacokinetic interaction between rifampicin and the once-daily combination of saquinavir and low-dose zidovudine in HIV-infected patients with tuberculosis. *J. Antimicrob. Chemother.* 59:690–697.
19. Sekar, V., R. Ryan, D. Schallie, A. Mazkewich, and J. Mrus. 2008. Pharmacokinetic profile of darunavir co-administered with low-dose zidovudine in treatment-experienced women and men with HIV infection: 4-week analysis in a substudy of the GRACE (gender, race and clinical experience) study, abstr. O16, p. 46. Abstr. Ninth Int. Workshop Clin. Pharmacol. HIV Ther., New Orleans, LA, 7 to 9 April 2008.
20. Tortajada, C., et al. 2005. Is the combination of pyrazinamide plus rifampicin safe for treating latent tuberculosis infection in persons not infected by the human immunodeficiency virus? *Int. J. Tuberc. Lung Dis.* 9:276–281.
21. Umeh, O. C., et al. 13 January 2011. Sex differences in lopinavir and ritonavir pharmacokinetics among HIV-infected women and men. *J. Clin. Pharmacol.* [Epub ahead of print.] doi:10.1177/0091270010388650.
22. Zhou, S., et al. 2004. Therapeutic drugs that behave as mechanism-based inhibitors of cytochrome P450 3A4. *Curr. Drug Metab.* 5:415–442.

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Chapter 3

The Protocol

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**The pharmacokinetics of lopinavir in South African HIV-infected volunteers
receiving rifampicin with adjusted doses of LOPINAVIR/ritonavir (600/150 mg
and 800/200 mg)**

APK.DDK - study

Version 5, dated 30/10/2009

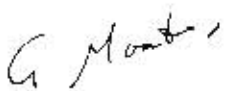
University of Cape Town

Authorized by:

Name: Dr Concepta Merry Role: Chief Investigator

Signature:  Date: 2 June 2009

Name: Prof Gary Maartens Role: Project Director

Signature:  Date: 2 June 2009

Name: Dr Helen McIlleron Role: National Principal Investigator

Signature:  Date: 2 June 2009

Protocol Title: The pharmacokinetics of lopinavir in South African HIV-infected volunteers receiving rifampicin with adjusted doses of LOPINAVIR/ritonavir (600/150 mg and 800/200 mg)

Protocol code: APK.DDK

INVESTIGATORS:

Dr Concepta Merry^{1,2}, Professor Gary Maartens², Dr Helen McIlleron²,
Dr Eric Decloedt¹, Professor Pete Smith², Professor David Back³, Prof David Burger⁴,
Professor Mats Karlsson⁵

INVESTIGATOR'S INSTITUTIONS:

¹ Department of Pharmacology, Trinity College Dublin

² Division of Clinical Pharmacology, University of Cape Town

³ Department of Pharmacology and Therapeutics, University of Liverpool

⁴ Radboud University Medical Center and Nijmegen University Center for Infectious Diseases

⁵ Division of Pharmacokinetics and Drug Therapy, Uppsala University

Data Safety and Monitoring Board (DSMB):

Dr W Burman (Denver Public Health, Colorado), Dr A Pozniak (Chelsea and Westminster Hospital, London), Prof P Waako (Makerere University, Kampala)

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University of Cape Town

INVESTIGATORS and CORE STAFF:

Dr Concepta Merry: Chief Investigator (Ireland)	Honorary Research Associate, Division of Clinical Pharmacology, University of Cape Town Dept of Pharmacology, Trinity College Dublin, St James's Hospital, Dublin 8, Ireland Tel: + 353 87 6539409 Fax: + 353 14730596 e-mail: cmerry@tcd.ie
Professor Gary Maartens: Principal Investigator	Division of Clinical Pharmacology, University of Cape Town, K45 Old Main Building, Groote Schuur Hospital, Observatory 7925, South Africa Tel: +27 21 406 6292 / +27 21 406 6008 Fax: +27 21 448 1989 e-mail addresses: gary.maartens@uct.ac.za; helen.mcilleron@uct.ac.za; peter.smith@uct.ac.za; marque.venter@uct.ac.za; wynand.smythe@uct.ac.za; eric.decloedt@uct.ac.za
Dr Helen Mcilleron: Project director	
Dr Eric Decloedt: Co-investigator	
Professor Pete Smith: Laboratory director	
Ms Marque Venter: Study coordinator	
Mr Wynand Smythe: Study Pharmacist	
Professor David Back: Expert advisor, UK	
Prof David Burger: Expert advisor, Netherlands	
Professor Mats Karlsson: Expert advisor, Sweden	

SUMMARY

There is an urgent need to investigate practical approaches to using antiretrovirals together with tuberculosis (TB) treatment. Administration of rifampicin with Kaletra® (lopinavir 400 mg/ritonavir 100 mg 12 hourly) reduces the lopinavir (LPV) pharmacokinetic parameters area under the curve and trough concentrations by approximately 75% and 99% respectively. A small study in healthy adults [La Porte 2004] found that doubling the dose of Kaletra® (LPV 800 mg/ritonavir [RTV] 200 mg), or giving additional RTV (LPV 400 mg/RTV 400 mg 12 hourly), ameliorates the rifampicin-mediated decrease in LPV concentrations. There is an urgent need to investigate these approaches in South African patients requiring combined treatment for TB and HIV.

We propose to study the steady state pharmacokinetics of LPV in 24 HIV-infected adult volunteers established on an antiretroviral regimen comprising LPV/RTV (in the film-coated tablet formulation Aluvia®) plus 2 NRTIs. Intensive blood sampling will be employed over a single dosing interval (12 hours) to study the pharmacokinetics of LPV at baseline, after which rifampicin will be added. After steady state has been achieved on rifampicin intensive sampling will be repeated. The pharmacokinetics will then be evaluated after increasing in the dose of LPV/RTV to 1.5 and 2 times the standard dose, respectively.

As multiple viral mutations are required to confer LPV resistance [Kempf 2001] and because we will only enroll participants who have virologic suppression, there should be no risk of resistance developing should the concentrations of LPV be low during the short period of the study. Participants will be carefully monitored for the development of toxicity during the administration of rifampicin with the antiretroviral therapy, by means of frequent alanine aminotransferase (ALT) and total serum bilirubin measurements.

Patients will be recruited at antiretroviral clinics in the Western Cape and admitted to the pharmacokinetic facility at the Division of Clinical Pharmacology, University of Cape Town and Groote Schuur Hospital for pharmacokinetic assessment. The study will be conducted in accordance with international (ICH) and local (National Department of Health) Good Clinical Practice Guidelines. The study is funded by the European and Developing Countries Clinical Trials Partnership. The University of Cape Town is the sponsor.

ABBREVIATIONS

AE	Adverse event
ALT	Alanine aminotransferase
ART	Antiretroviral treatment
ARV	Antiretroviral
AUC	Area under the drug concentration-time curve
C_{max}	Maximum / peak concentration
C_{min}	Minimum concentration
CRP	C-reactive protein
CYP	Cytochrome P450 enzyme
DSMB	Data safety and monitoring board
HAART	Highly active antiretroviral treatment
HBVsAg	Hepatitis B virus surface antigen
HCV Ab	Hepatitis C virus antibody
HIV	Human immunodeficiency virus
IQR	Inter-quartile range
LPV	Lopinavir
MCC	Medicines Control Council of South Africa
NCA	Noncompartmental analysis
NNRTI	Nonnucleoside reverse transcriptase inhibitor
NRTI	Nucleoside reverse transcriptase inhibitor
PI	Protease inhibitor
PK	Pharmacokinetic(s)
RIF	Rifampicin
RTV	Ritonavir
SAE	Serious adverse event
TB	Tuberculosis
TSB	Total serum bilirubin
UCT	University of Cape Town

1. STUDY RATIONALE

There is an urgent need to evaluate highly active antiretroviral (ARV) treatment regimens that are compatible with rifampicin-based antitubercular treatments. Combined treatment is life-saving for tuberculosis patients with advanced HIV-related immunosuppression. Co-administration of ARV and antitubercular treatment regimens is occurring frequently in countries where the burdens of HIV and TB are high. However, major concerns of using HIV and TB treatments together include pharmacokinetic (PK) drug-drug interactions, combined regimen toxicity and the development of immunopathological reactions. The optimal approaches to co-administration of TB and HIV treatment regimens have not been adequately evaluated in patient populations. Moreover, the optimization of co-treatment strategies is all the more important where target drug concentration measurement is not generally available.

Rifamycin-based regimens are recommended for the treatment of TB, especially in the presence of HIV-infection. Rifampicin is a potent inducer of cytochrome P450 (CYP) isoenzyme 3A and also causes several fold increases in the expression of CYP2B6, and p-glycoprotein (an important transmembrane efflux protein and product of the MDR-1 gene), amongst other enzymes of importance for PK [Rae 2001]. Thus rifampicin results in important reductions in the concentrations of protease inhibitors (PIs) and nonnucleoside reverse transcriptase inhibitors (NNRTIs). Although rifabutin causes less enzyme induction (and some guidelines recommend that it should replace rifampicin in patients receiving concurrent LPV/RTV [US DHSS 2006]), it is expensive and its dosing is complex with PIs which inhibit rifabutin metabolism; it is currently not accommodated within the TB control programs of most countries with a high burden of TB/HIV.

Guidelines for managing the interactions between the rifamycins and antiretrovirals [US-DHHS 2006; SA Dept. of Health National (2004)] are based primarily on drug interaction studies in healthy volunteers and expert opinion. Research is urgently needed to better characterize these interactions in the relevant patient populations with HIV-associated tuberculosis, and to understand the pharmacodynamic consequences of the resultant changes in serum concentrations of antiretroviral drugs.

A small study [La Porte 2004] amongst adult healthy volunteers investigated the approach of adjusted doses of LPV/RTV to counteract the enzyme induction caused by concomitant rifampicin. Volunteers were established on twice daily LPV/RTV in doses of 400/100 mg for several days before the introduction of rifampicin and escalation of the PI doses to reach a steady state on dosing with rifampicin 600 mg once daily plus 12 hourly doses of RTV 400 mg in conjunction with LPV 400 mg (arm 2), or double doses of Kaletra®: LPV/RTV 800/200 mg (arm 1). As shown in the Table 1, both approaches offset the effect of rifampicin-mediated induction of LPV (Table 1).

Table 1: The pharmacokinetic measures for lopinavir when given 12 hourly in combination with ritonavir and a daily dose of rifampicin 600 mg in healthy volunteers [La Porte 2004]. The point estimate (90% CI) of the geometric mean ratio (GMR) is also given for co-administration of Kaletra® and rifampicin \pm ritonavir / standard doses of Kaletra® in the same subjects.

LPV/RTV:	N	C _{max}	AUC	C _{min}
800/200 mg (arm 1)	10	13.8 \pm 4.89 mg/L GMR: 1.02 (0.85-1.23)	104.5 \pm 46.86 mg.h/L GMR: 0.84 (0.64-1.10)	5.1 \pm 4.17 mg/L GMR: 0.43 (0.19-0.96)
400/400 mg (arm 2)	9	11.5 \pm 3.07 mg/L GMR: 0.93 (0.81-1.07)	100.7 \pm 26.81 mg.h/L GMR: 0.98 (0.81-1.17)	5.9 \pm 2.73 mg/L GMR: 1.03 (0.68-1.56)

The study was underpowered to assess differences between the 2 dosing approaches in combination with rifampicin. Although the trough concentration (C_{min}) for arm 1 was reduced in comparison to standard Kaletra® doses without rifampicin, the difference between the mean C_{min} for the 2 arms was less obvious (*arm 1*: 5.1 mg/L \pm 4.17; vs. *arm 2*: 5.9 mg/L \pm 2.73 mg/L) and the trough concentrations were above the recommended minimum target concentration of 1 mg/L for protease inhibitor naïve patients [La Porte 2006]. The study was not designed to establish safety of the combinations. However, 28% of the volunteers discontinued due to liver enzyme abnormalities. The adverse events tended to be less frequent in the group given LPV/RTV 800/200 mg. Based on this study, dosing approaches have been recommended when it is necessary to use the combination of LPV/RTV and rifampicin-based TB treatment (with the caution that patients are closely monitored and that further evidence of the safety and efficacy of the approaches are required).

The approach of using additional RTV with Kaletra® (LPV/RTV 400/400 mg) adds complexity to the combined dosing regimen; increasing the risk of errors in drug supply, prescribing and dispensing. Moreover, RTV is poorly tolerated in high doses. These factors favour the approach of increasing the dose of Kaletra® (with LPV:RTV in a ratio of 4:1) without adding extra RTV separately. The approach needs to be evaluated in an adequately powered study amongst the relevant patient populations; the existing evidence is based on a small underpowered study in healthy Caucasian male volunteers who are genomically, environmentally and behaviorally different to their African counterparts.

The film-coated tablet formulation of LPV/RTV (to be marketed as Aluvia® in South Africa) is less susceptible than the capsule to food effects and results in more predictable systemic LPV concentrations [Kaletra® package insert 2007]. The film-coated tablet has replaced the capsule in many countries; and will shortly become available in South Africa.

LPV/RTV is commonly associated with diarrhoea, nausea and vomiting; the lipodystrophy syndrome is less common; and pancreatitis, hyperglycaemia, ketoacidosis, diabetes and hepatitis are rare. Hypersensitivity reactions may also occur.

Rifampicin administration is commonly associated with elevated serum transaminase concentrations. Progression to liver injury is unpredictable and clinical hepatitis is rare but may be fatal. The use of other potentially hepatotoxic drugs may enhance the risk of

hepatitis developing. Inhibition of bilirubin excretion may cause jaundice on initiation of rifampicin, but it usually clears with ongoing therapy. Gastrointestinal effects include nausea and vomiting, anorexia, mild abdominal discomfort and diarrhoea. Taking the drug with food may alleviate the symptoms, although administration on an empty stomach is recommended for optimal absorption. Hypersensitivity reactions are mainly associated with intermittent or discontinuous therapy and include a 'flu-like' syndrome with fever, urticaria, haemolysis, eosinophilia, thrombocytopenia, leucopenia, interstitial nephritis and acute tubular necrosis. CNS effects such as drowsiness, headache, mental confusion and muscular weakness are described. Rifampicin colours urine, tears and other body fluids reddish-orange to reddish-brown; staining of contact lenses may occur.

Concerns relating to toxicity of PI – rifampicin combinations arise largely from two recent studies in healthy volunteers [Grange 6th International Workshop on Clinical Pharmacology of HIV Therapy 2005; D. Burger, 8th International Workshop on Clinical Pharmacology of HIV Therapy 2007]. These 2 studies were terminated due to the high incidence of severe hepatotoxicity in the participants who received increased doses of protease inhibitors (saquinavir with RTV, and LPV/RTV, respectively) after several daily doses of rifampicin. Participants who took LPV/RTV before rifampicin had less hepatotoxicity. It is not known why treatment sequence should be an important determinant of hepatic damage, but it is speculated that prior induction of the liver enzymes by rifampicin, may give rise to high concentrations of toxic intermediate metabolites when LPV/RTV is introduced. The use of adjusted dose protease inhibitors with rifampicin appears to be well tolerated in patients, but this has been poorly documented.

These concerns, together with the fact that the reported trough concentrations of LPV are well above the lower limit of the recommended range, and wide variability in the LPV concentrations between subjects, support the approach of studying LPV concentrations using 1.5 X in addition to 2 X the usual dose of Kaletra® in patients receiving rifampicin, and frequent monitoring of transaminase concentrations during the period of rifampicin administration.

Earlier this year, 6 April 2009, the FDA issued a warning that lopinavir/ritonavir prolongs the PR interval in some patients; and although causality of lopinavir/ritonavir could not be established, postmarketing cases of QT interval prolongation and torsade de pointes have been reported with Kaletra® use.

Pharmacokinetic evaluation:

Intensive pharmacokinetic sampling will be used to characterize the pharmacokinetics using noncompartmental analysis (NCA). This approach allows characterization of the pharmacokinetic profiles of the drugs in a relatively small number of patients with rapid statistical analysis and reporting.

Pharmacokinetics of Kaletra® in standard recommended doses (400/100 mg) in HIV-infected patients:

After multiple 12 hourly doses of the capsule form of Kaletra® a mean peak LPV concentration (C_{max}) of 9.8 mg/L (SD: 3.7) was achieved approximately 4 hours after dosing. The steady state trough concentration was 7.1 mg/L (SD: 2.9) and the AUC during a

dosing interval was 92.6 (SD: 36.7). Plasma concentrations of LPV and RTV after administration of two 200/50 mg tablets are similar to three 133.3/33.3 mg capsules under fed conditions, but with less pharmacokinetic variability (Kaletra® package insert). Unlike that following administration of the capsule, the bioavailability of LPV in the tablet formulation is not substantially altered by concomitant food ingestion: With a high fat (~56%) meal and a medium fat (~25% fat) the AUC was increased by 19% and 27% respectively, in comparison to taking it under fasting conditions. The tablet formulation of LPV/RTV (Aluvia®) can therefore be taken with or without food.

Proposed study:

We propose to study the steady state pharmacokinetics of LPV in 24 HIV-infected adult volunteers established on an antiretroviral regimen comprising LPV/RTV plus 2 NRTIs. Intensive blood sampling will be employed over a single dosing interval (12 hours) to study the pharmacokinetics of LPV on 4 sequential occasions:

- 1) at baseline (LPV 400 mg/RTV 100 mg 12 hourly),
- 2) after steady state has been achieved with the addition of rifampicin (600 mg daily for 6 days),
- 3) after steady state has been achieved with a dose of LPV/RTV 1.5 times the standard dose (600 mg/150 mg, 12 hourly) with rifampicin, and
- 4) after steady state has been achieved with a dose of LPV/RTV 2 times the standard dose (800 mg/ 200 mg, 12 hourly) with rifampicin.

Rifampicin will then be stopped and the dose of LPV/RTV returned to normal. The pharmacokinetics of LPV will be compared between the 4 sampling occasions. Trough concentrations of LPV will be compared to the recommended minimum target concentration (1 mg/L).

Secondary objectives include the documentation of adverse events and laboratory abnormalities in enrolled patients.

The study will be submitted to the South African Medicines Control Council and the Research Ethics Committee of the University of Cape Town for their approval, and will be conducted in accordance with ICH GCP (1997), South African regulatory requirements and the Declaration of Helsinki.

2. STUDY OBJECTIVES

2.1 Using noncompartmental analysis (NCA), to describe the steady state pharmacokinetics of LPV and RTV over a 12 h dosing interval in 24 HIV-infected volunteers treated with:

2.1.1 LPV/RTV in standard doses (400 mg LPV/100 mg RTV) 12 hourly together with 2 NRTIs (Baseline measurement; PK1)

2.1.2 LPV/RTV in standard doses (400 mg LPV/100 mg RTV) 12 hourly together with 2 NRTIs and rifampicin 600 mg daily (PK2)

2.1.3 LPV/RTV at 1.5 X the standard doses (600 mg LPV/150 mg RTV) with 2 NRTIs AND rifampicin 600 mg daily (PK3)

2.1.4 LPV/RTV at 2 X the standard doses (800 mg LPV/200 mg RTV) with 2 NRTIs AND rifampicin 600 mg daily (PK4)

2.2 To compare the pharmacokinetics of LPV and RTV between PK1, PK2, PK3 and PK4.

2.3 To document and evaluate adverse events and laboratory abnormalities in all patients throughout the study period.

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3.1 STUDY DESIGN

The steady state pharmacokinetics of LPV and RTV will be evaluated in a cohort of South African HIV-infected volunteers under 4 sequential treatment conditions in steady state: LPV/RTV in standard doses (400 mg/100 mg) 12 hourly together with 2 NRTIs (standard 2nd-line ARV regimen) without rifampicin (PK1; DAY1); LPV/RTV in standard doses 12 hourly together with 2 NRTIs AND rifampicin (PK2; DAY8); 1.5 X the usual dose of LPV/RTV (600 mg/150 mg) 12 hourly together with 2 NRTIs AND rifampicin (PK3; DAY15); and 2 X the usual dose of LPV/RTV (800 mg/200 mg) 12 hourly together with 2 NRTIs AND rifampicin (PK4; DAY22).

3.2 STUDY METHODS

3.2.1. Study participants:

HIV-infected volunteers established on 400mg LPV/100 mg RTV plus 2 NRTIs will be recruited from ARV clinics within the Western Cape province.

3.2.1.1. Inclusion criteria:

- HIV-infected adults (≥ 18 yr)
- Established on an antiretroviral regimen comprising LPV 400 mg/RTV 100 mg and 2 NRTIs > 6 months
- Undetectable viral load (< 50 viral copies/mL); confirmed by VL test within 12 weeks of the study
- ALT (within 1 week of enrolment) < 1.5 X the upper limit of normal
- Recent (within 12 weeks of enrolment) Hepatitis B virus surface antigen negative and HCV Ab negative
- Normal ECG at a screening visit
- Normal serum potassium at a screening visit
- Medically stable*

3.2.1.2. Exclusion criteria:

- Previously failed to attain or maintain virological suppression on a protease inhibitor-containing regimen.
- Known to have chronic renal, hepatic or GIT disease that may interfere with the pharmacokinetics of the drugs studied.
- Known to have cardiac disease that may increase the risk for developing cardiac conduction abnormalities.
- ECG changes consistent with a prolonged PR interval (> 0.20 s) and QT prolongation as identified by using a QT correction formula. QT interval with Fridericia's correction > 480 ms.
- Hypo or hyperkalaemia, a family history of Long QT syndrome or on medication that may prolong the QT/QTc interval.

- Fasting cholesterol >7.77 mmol/L (> 300 mg/dL; grade 3 or 4¹), fasting triglyceride > 8.49 mmol/L (> 750 mg/dL; grade 3 or 4) or abnormal glucose measurements at baseline or 6 monthly checks (routine tests as per National Guidelines).
- Alcohol consumption in excess of 2 units/day or 14 units/week.
- Tuberculosis (TB) will be excluded by a structured symptom questionnaire. This was found to be 100% sensitive in excluding TB in patients with advanced HIV disease in Cape Town (Mohammed 2004). Patients with any of these validated symptoms² will not be enrolled.
- Receiving drugs other than the study medication known to potentially induce or inhibit CYP3A4 or alter the PK of LPV; at the discretion of the PI*.
- Receiving drugs (e.g. Oral contraceptive) other than the study drugs whose PK is known to be altered (with potentially important consequences) by Kaletra®*.
- Known or suspected pregnancy*.
- Women of child-bearing potential who are not using a recognized form of contraception*; An injectable progestogen-based method of contraception is recommended for women requiring contraception.

**Withdrawal from the study will be considered should these criteria no longer be satisfied*

3.2.2. Study treatments:

3.2.2.1. Formulations:

LPV/RTV: Aluvia, Abbott
Film-coated tablet: LPV 200 mg co-formulated with RTV 50 mg
(not currently registered in South Africa)

Rifampicin: Rimactane®, Sandoz
Tablet formulation: Rifampicin 600 mg
(SA National Registration number: H/20.2.3/68-69).

Patients will continue their regular treatment including dual NRTI backbone and other medications as prescribed and supplied by their regular health care provider.

3.2.2.2. Study treatment schedule:

DAY -5 to DAY 1: 400 mg LPV/100 mg RTV 12 hourly

DAY 2 to DAY 8 (7 days): 400 mg LPV/100 mg RTV 12 hourly AND rifampicin 600 mg daily

DAY 9 to DAY 15 (7 days): 600 mg LPV/150 mg RTV 12 hourly AND rifampicin 600 mg daily

DAY 16 to DAY 22 (7 days): 800 mg LPV/200 mg RTV 12 hourly AND rifampicin 600 mg daily

DAY 23: Resume standard of care treatment (with 400 mg LPV/100 mg RTV 12 hourly)

¹ Division of AIDS table for grading the severity of adult and pediatric adverse events (DAIDS AE grading table); December 2004.

² Weight loss > 2.5% in 4 weeks; cough > 2 weeks; nightsweats > 2 weeks; fever > 2 weeks.

The treatment durations are based on the assumptions that the inducing effect of rifampicin will take more-or-less 6 days to reach a maximum and the same time to wane.

3.2.2.3. Treatment procurement and storage:

It is anticipated that Aluvia® will be registered in South Africa at the time of the study. It will be procured through an authorized commercial supplier.

Rimactane® (Sandoz; SA National Registration number: H/20.2.3/68-69) will be procured through an authorized commercial supplier.

The drugs will be stored at room temperature in a locked area with access restricted to designated study personnel.

An inventory of receipt and disposition of all study drugs will be kept by the study pharmacist.

3.2.2.4. Treatment packaging and labeling:

The treatments will be packed within the Division of Clinical Pharmacology, UCT, by the study pharmacist. Separate packages will be prepared for each participant containing rifampicin and lopinavir/ritonavir, respectively. The study treatment will be packaged for weekly dispensing at the study visits on Day -5, Day 8, Day 15 and Day 22 (see study schedule – 4.1). Each package of dispensed medication will be labelled with the study number, the individual study participant's ID, the study days that the medication is to be taken, the generic and trade names of the products (manufacturer) with batch numbers and expiry dates, the investigators name (contact number), and "for study use only; keep in a safe place out of reach of children".

3.2.2.5. Study treatment dispensing, administration and adherence:

The study pharmacist will dispense the of study medication and keep records thereof.

Patients will be given a week's supply of study medication to take at home on Day -5, Day 1, Day 8, and Day 15. Together with the medication the participant will be given a self-report treatment card to record all doses and drug taking times for the study treatment dispensed. Patients will also be requested to bring the remaining study treatment and all their other medicines to the ward for PK sampling admissions.

On the days of pharmacokinetic sampling, the dose administration will be carefully observed by the investigators / designated assistant and a mouth check performed. The exact time of dose administration on PK days will be recorded in the CRF.

3.2.3. Pharmacokinetic (PK) assessment:

PK sampling schedule (DAYS 1, 8, 15 and 22)			
Scheduled time after drug dosing for blood sampling			
1.	pre-dose	-0.5 to 0 h	(4 ml)
2.	1.5 h	± 1 min	(4 ml)
3.	2 h	± 1 min	(4 ml)
4.	2.5 h	± 1 min	(4 ml)
5.	3 h	± 1 min	(4 ml)
6.	4 h	± 1 min	(4 ml)
7.	5 h	± 1 min	(4 ml)
8.	6 h	± 1 min	(4 ml)
9.	8 h	± 1 min	(4 ml)
10.	12 h	± 1 min	(12 ml)
total volume=48 ml			

n=24

The exact (to the minute) times of sampling must be recorded in the study documents. If samples are deviate from the scheduled time, the reason should be noted in the CRF.

Intensive samples should be drawn at the times (after treatment administration) indicated in Table 2. Pre-dose samples should be taken before the lopinavir/ritonavir dose on the morning of PK sampling.

Sample labelling: Each sample will be labelled with the participant's study code, the sampling occasion (1 for PK1; 2 for PK2; or 3 for PK3) and the number of the sample (1 [pre-dose] to 10 [at 12 h]).

Eg. A04K – 1 – 7 will be used to label the 7th (5-hour) sample taken at the PK1 (DAY 1) assessment from participant A04.

E.g. A23K – 3 – 1 will be used to label the pre-dose sample taken at PK3 (DAY 22) from participant A23.

3.2.4. Determination of drug concentrations:

LC-MS/MS: Validated liquid chromatography – tandem mass spectroscopy (LC-MS/MS) methods will be used to determine LPV and RTV concentrations in the plasma samples in the Division of Pharmacology's laboratory, which is ISO17025 accredited for this purpose. Concentrations below the limits of quantification will be reported as such.

Plasma (LPV, RTV): 4 ml blood samples will be collected in heparinized tubes. Within a maximum of 1 hour from the time of sampling, the blood specimens will be centrifuged to separate the plasma (10 minutes at 3000 rpm). Blood samples will be kept on melting ice prior to separation. From each sample, 2 x 0.25 ml aliquots of plasma will be transferred to into duplicate labelled polypropylene tubes and for stored at -80°C in the laboratory (Division of Clinical Pharmacology, UCT) until drug concentration determination. The label

on each polypropylene storage tube will be identical to the label on the blood sample (see above) – and include the subject number, sampling occasion, and the sampling time.

The results of LPV concentrations taken at PK1 will be analysed within 2 weeks of collection and will be made available to the attending clinician.

3.2.5. Safety monitoring:

Adverse events (AEs) will be recorded at screening and on DAY 1, DAY 8, DAY 15, DAY 22 and DAY 29. A standardized assessment form will be used.

ALT and total serum bilirubin will be monitored at screening and then 3 x weekly from the day before rifampicin is started until the end of the study period (screening, DAYS 1, 4, 6, 8, 11, 13, 15, 18, 20, 22, 25 and 28).

AEs will be graded according to the US Division of AIDS table for grading the severity of adult and paediatric adverse events (2004) and managed in accordance with the National Treatment Guidelines. In the event of a grade 3 or 4 AE thought to be related to the study intervention, the subject will be immediately withdrawn from the study and the DSMB informed. In the event of unexplained abnormal laboratory test values, the tests should be repeated immediately. After the initial AE/serious adverse event (SAE) report, the investigator should take all appropriate measures to ensure the safety of the patient until resolution or until consolidation of the patient's condition.

SAEs will be reported to the Data Safety and Monitoring Board, the Research Ethics Committee and to the SA Medicines Control Council within 2 working days.

The study will be stopped should 2 or more of the first 5 participants experience a SAE, or if 3 participants (of the enrolled cohort) experience a SAE.

3.2.6. Other laboratory tests:

Haematology, chemistry and viral loads will be performed by standard operating procedures in the laboratories of the NHLS (National Health Laboratory Services).

3.2.7. Data and recording and reporting:

- All data will be recorded in the study specific case record form of each participant.
- Double data entry will be used to transcribe the data for analysis into an electronic data base, except in the case of data that is generated electronically (e.g. LC-MS/MS results).

3.2.8. PK analysis:

- Plasma PK data will be analysed using NCA to determine the following PK measures for LPV and RTV:

C_{min} (pre-dose sample concentration)
C₁₂ (concentration 12 h after observed dose)
C_{max} (peak plasma concentration)
T_{max} (the time to reach C_{max} after drug ingestion)
AUC(0-12) (the area under the concentration-time curve from 0-12 hours after drug ingestion)
Half – life

NCA will be performed using the software package WinNonlin (Pharsight Corporation, US). Drug concentrations below the limit of quantification will be treated as 'missing data'.

3.2.9. Statistics:

- The proportions of trough and 12 h concentrations < lower limit of the recommended range (1 mg/L) will reported for PK1, 2, 3 and 4, respectively. Comparisons between the PK assessments will be made using Chi-2 test.

- The LPV and RTV PK measures generated by NCA from the data in intensively sampled patients with and without TB treatment will be compared (using paired t-tests or equivalent nonparametric analyses for skewed data). The geometric mean ratios (90% confidence intervals) will be used to compare the C_{max} and AUC(0-12) at PK2, PK3 and PK4, respectively, with PK1. Regression analyses will be used to identify potential significant covariate factors that might affect LPV or RTV concentrations.

3.2.10. Sample size justification:

The sample size approximates that usually employed for drug interaction studies of this nature. It should be sufficient to generate reliable estimates of the geometric mean ratios of the drug concentrations at PK2, PK3 and PK4, respectively, compared to LPV and RTV concentrations at PK1.

3.3. SELECTED ETHICAL AND REGULATORY ASPECTS

3.3.1. Informed consent:

The investigator or a designated study team member will fully explain the nature, conditions and consequences of the study. A competent translator, familiar with the study will assist in the informed consent process when necessary. Subject information and consent documents will be available in Xhosa, Afrikaans and English. A witness independent of the study will countersign the consent documents of illiterate patients. Each participant will be given a copy of the signed consent document. No patient will be enrolled prior to or without their written informed consent.

3.3.2. Data management and storage:

Individual source document files for study related source documents (screening notes, adverse events reports, blood test result reports, adherence questionnaire documents etc.) will be kept at the study site. In addition, a case record form (CRF) will be compiled for each participant for collection of subject specific details, study-related blood test results (apart from drug concentrations), drug dosing details and the exact times of PK sampling. The raw data relating to the drug concentrations will be kept in a study specific document. All study related documents will be stored for at least 10 years following study completion. A database will be populated with the relevant data using a system of double data entry (except for data imported electronically from the mass spectrometer). The duplicate plasma samples will be kept at -80°C until one year after study completion.

3.3.3. Compensation for participation:

Each participant will be compensated to cover travel costs and inconvenience on a pro-rata basis: For each intensive sampling admission (2 nights and a day) R310; and for each short study-specific visit, an amount of R50.

3.3.4. Withdrawal:

Participants are free to withdraw from the study at any stage, without prejudice to them or affecting their rights to care in any way.

Withdrawal will be indicated in the event of Grade 3 or 4 adverse events related to the study intervention.

Participation in the clinical trial may be discontinued should the attending clinician deem it in the best interest of the patient, if there is poor compliance with visits or non-adherence to study medication (see also footnote to exclusion criteria: *"*Withdrawal from the study will be considered should these criteria no longer be satisfied."*)

For each patient withdrawn from the study, a CRF must be completed at the last visit performed. The investigator should make every effort to re-contact and to identify the reason why the patient failed to attend the visit and to determine his/her health status.

3.3.5. Insurance:

The University of Cape Town is the sponsor of the study and the study will be conducted under the auspices of the University. The University's insurance policies covering all persons participating in Clinical Trials research apply; compensation will be paid in accordance with the policies, when on the balance of probabilities, the injury was attributable to the administration of a medicinal product or trial procedure provided for by the protocol that would not have occurred but for the inclusion of the patient in the trial. The Guidelines for Good Clinical Practice in the conduct of Clinical Trials in human participants in South Africa (Department of Health 2000) will be followed. The insurance will cover the costs of any medical care required for study-related events, the affected participants will receive free

medical care (through the public or private sectors) for the treatment of the adverse event until its resolution. In addition, the University of Cape Town subscribes to insurance policies which include professional liability (policy number 20062796-05) and personal accident (policy number: 148901/C0206) which also apply to researcher and research participants involved in research conducted under the auspices of the University.

3.3.6. Ethical and regulatory considerations:

The risks and inconveniences of the study will be fully explained to each participant. No subject will be enrolled in the study before they have been fully informed about the study verbally and by means of a subject information document, with the aid of a translator if necessary, and signed consent has been obtained on the study specific consent form.

Safety monitoring will limit harm amongst participants. The DSMB will be fully informed of any grade 3 or 4 adverse events, and will act to stop the study should they deem the risks greater than the potential benefit of the study.

The study documents will be submitted to the Medicines Control Council (MCC) of South Africa and to the Research Ethics Committee of the University of Cape Town. Approval of the protocol and any amendments will be obtained prior to implementation.

The study will be conducted in accordance with the Declaration of Helsinki 2008, international guidelines for Good Clinical Practice (ICH 1997) and according to national guidelines of Good Clinical Practice [National Department of Health 2006]

4. 1. Study schedule:

Note: Enrolment and screening will take place from DAY-30 to DAY-6. The relevant procedures are not included in the table above but are described in section 4.2, below.

4.2.1. Potentially eligible patients will be enrolled after written informed consent has been obtained.

4.2.2. A log will be kept of all enrolled patients. The date of enrollment, the date of screening, the participant's study code, and the date of study completion/withdrawal (as appropriate). The reasons for withdrawal/ nonparticipation will also be documented in the study log.

- date of birth, sex
- weight, height
- WHO stage on starting HAART
- routine baseline tests (routine tests on starting Kaletra®-based HAART in accordance with National ARV Treatment Guidelines): viral load, CD4+ cell count, ALT, FBC, glucose, triglycerides and cholesterol.
- results of last routine (6 monthly) measurements of viral load, CD4+ cell count, ALT, FBC, glucose, triglycerides and cholesterol, if applicable.
- adverse events, other antiretroviral drugs and concomitant medicines form
- electrocardiogram (ECG)

- Viral load (VL) - if the most recent VL was sent > 12 weeks before enrolment
- ALT if the most recent ALT test was > 1 week before enrolment
- Serum potassium will be done within 1 week of enrolment
- HBVsAg and HCV Ab - if not done within the 12 weeks before enrolment

4.2.4. A week's supply of the TABLET formulation (Aluvia®) will be dispensed to the participant to take from DAY-5 to DAY1 (at PK1). Together with the medication the

participant will be given a self-report treatment card to record all doses and drug taking times for the study treatment dispensed. Participants whose enrolment visit occurs prior to DAY -5 will be contacted by telephone on DAY-6, to remind them to switch from their standard Kaletra® to the tablet formulation; alternatively an appointment will be made for them to attend the study site to collect the tablet formulation on DAY -7 or DAY -6.

4.2.5. On DAY -6 or DAY -5, participants will be reminded to contact the study doctor immediately, should he/she experience any new symptoms or exacerbation of symptoms during the study period. He/she will be asked to inform any other health care providers they might encounter during the study, of their participation, and to urge the health care provider to contact the study doctor should they be concerned that any of signs or symptoms of the patient may be related to the study or if they wish to alter the patient's medication in any way.

4.4. PK sampling admissions (PK1, PK2, PK3, PK4):

4.4.1 Participant reminder:

The study nurse/coordinator will contact participants during the week prior to the PK assessment visits to final arrangements to ensure that the participant arrives at the study ward (J51 Old Main Building, Groote Schuur Hospital) at about 19h00 on the evening prior to the PK sampling days. The study nurse/coordinator will also remind the participant to bring with him/her any outstanding study treatments, his/her regular medication and his/her self-report treatment card.

4.4.2. Deferment of sampling:

PK sampling will be deferred and another PK assessment day schedules if:

1) The participant vomited after taking his/her ARV treatment the that morning, has acute diarrhea, or is medically unstable

OR

2) If the participant has been less than 100% adherent to treatment during the previous 3 days (as documented on the patient's self-reported treatment record).

4.4.3. Admission procedures and refreshments:

Participants will be admitted to the ward. An adverse events form will be filled out for each participant and his/her blood pressure, pulse and temperature recorded.

After a light standardized meal. Participants will fast overnight until 2 hours after the morning treatment administration, but water will be allowed freely except for the hour before and the hour after the morning treatment administration.

A light standardized breakfast will be served 2 hours after treatment administration, and further light standardized refreshments will be provided until discharge the following morning. Alcohol, grapefruit juice, caffeine and black pepper will be avoided from admission until 12 h after dose administration.

Patients will have the choice of going home after the 12 h blood sample, or staying overnight in the study ward until the next morning.

4.3.4. Blood sampling:

A cannula will be inserted into a suitable arm vein for obtaining 4 ml venous blood samples the times indicated in section 3.2.3. Lithium/heparin blood collection tubes will be used.

Plasma samples will be prepared from samples at all 10 sampling times (section 3.2.4).

The exact times (to the nearest minute) of blood sampling will be noted in the CRF.

4.3.5. Dose administration::

Immediately (within 30 minutes) after the pre-dose sampling, the study treatment will be given under the careful observation of a member of the study team (section 3.2.2.5). The exact time that the treatment is taken will be recorded in the case record form by the investigator or designated assistant.

4.3.6. Data collection at PK sampling visits:

- The previous week's study treatment doses and times (the self-reported treatment card will be collected)
- Adverse events and concomitant medication form will be completed
- ALT*, total bilirubin, (clotted tube; \pm 4 ml)
- FBC (EDTA tube; \pm 4 ml)
- At PK1: creatinine, albumin and C-reactive protein (clotted tube; \pm 4 ml)

** ALT and bilirubin results will be obtained before the evening dose of study treatments.*

4.5. Additional safety data:

In addition to the measurements on PK sampling days (Days 1, 8, 15 and 22), ALT will be measured on Days 4, 6, 11, 13, 18, 20, 25 and 28. Asymptomatic participants with ALT elevations $> 1.5 \times$ the upper limit of the normal range will be asked to present for ALT measurements every second day and will be contacted telephonically on a daily basis until resolution to below $1.5 \times$ the upper limit of normal. Should patients be symptomatic, they will be requested to present urgently at the study facility or any other appropriate facility for evaluation.

Participants with adverse events will be closely monitored until resolution. Grade 3 or 4 adverse events will necessitate withdrawal of the study treatments.

An ECG investigation will be repeated at Day 15 (before increasing the dose to double the dose of Aluvia[®]) and at Day 22 (to determine ECG changes on double dose Aluvia[®]). This will indicate ECG changes with the dose escalation. Not only will this address any safety concerns, but will add valuable safety information to Aluvia[®].

6. REFERENCES

Bonfanti P, Valsecchi L, Parazzini F, et al. Incidence of adverse reactions in HIV patients treated with protease inhibitors: a cohort study. J Acquir Immune Defic Syndr 2000; 23: 236-45.

Centers for Disease Control. Updated guidelines for the use of rifamycins for the treatment of tuberculosis among HIV-infected patients taking protease inhibitors or nonnucleoside reverse transcriptase inhibitors. Available from: http://www.cdc.gov/nchstp/tb/tb_hiv_drugs/toc.htm. Accessed 14 December 2005.

Grange S, Schutz M, Schmitt C, Riek M, Gaudeul-Ehrhart E. Unexpected hepatotoxicity observed in a healthy volunteer study on the effects of multiple dose rifampicin on the steady-state pharmacokinetics of ritonavir-boosted saquinavir and vice versa [abstract no. 35]. 6th International Workshop on Clinical Pharmacology of HIV therapy, Quebec, Canada 2005.

Guidelines for good clinical practice in the conduct of clinical trials in human participants in South Africa. Clinical Trials Guidelines 2000. Department of Health, Republic of South Africa.

Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents. Panel on Clinical Practices for Treatment of HIV Infection convened by the US Department of Health and Human Services (DHHS). *October 10, 2006*.

ICH Harmonized Tripartite Guideline for Good Clinical Practice. © ICH Secretariat 1996.

Kappelhoff BS, Crommentuyn KML, De Maat MMR, Mulder JW, Huitema1 ADR, Beijnen JH. Practical guidelines to interpret plasma concentrations of antiretroviral drugs. *Clin Pharmacokinet* 2004; 43 (13): 845-853.

Kempf DJ, Isaacson JD, King MS, Brun SC, Xu Y, Real K, Bernstein BM, Japour AJ, Sun E, Rode RA. Identification of genotypic changes in human immunodeficiency virus protease that correlate with reduced susceptibility to the protease inhibitor lopinavir among viral isolates from protease inhibitor-experienced patients. *J. Virol* 2001; 75:7462–7469.

La Porte CJL, Colbers EPH, Bertz R, et al. Pharmacokinetics of adjusted-dose lopinavir-ritonavir combined with rifampin in healthy volunteers. *Antimicrob Agents Chemother* 2004; 48:1553–60.

La Porte CJL, Back DJ, Blaschke T, Bouche CAB, Fletcher CV, Flexner C, Gerber JG, Kashuba ADM, Schapiro J, Burger DM. Updated guideline to perform therapeutic drug monitoring for antiretroviral agents. *Reviews in Antiviral Therapy* 2006; 3:4-12.

Le Guellec S, Veillon S, Arsac P, et al. High Lopinavir plasma concentrations are associated with changes in lipid levels at M6 in HIV-infected patients [abstract 44]. 6th International Workshop on Clinical Pharmacology of HIV therapy, Quebec, Canada 2005.

Lopinavir/Ritonavir (Kaletra) [package insert]. North Chicago (IL): Abbott Laboratories, 2000.

Mohammed A, Ehrlich R, Wood R, Cilliers F, Maartens G. Screening for tuberculosis in adults with advanced HIV infection prior to preventive therapy. *International Journal of Tuberculosis and Lung Disease* 2004;8:792-5.

Moreno S, Podzamczar D, Blazquez R, et al. Treatment of tuberculosis in HIV-infected patients: safety and antiretroviral efficacy of the concomitant use of ritonavir and rifampin. *AIDS* 2001; 15:1185-7.

National Antiretroviral Treatment Guidelines. National Department of Health South Africa 2004.

Rae JM, Johnson MD, Lippman ME, Flockhart DA. Rifampicin is a selective, pleiotropic inducer of drug metabolism: studies with cDNA and oligonucleotide expression assays. *J. Pharmacol Exp Ther* 2001; 299:849-57.

Sankatsing SUC, Beijnen JH, Schinkel AH, Lange JMA, Prins JM. P Glycoprotein in Human Immunodeficiency Virus Type 1 Infection and Therapy. *Antimicrobial Agents and Chemotherapy* 2004; 8(4): 1073-1081

Woo. J, W. C, R. C, Chan S, A. C, K. C. In Vitro Protein Binding Characteristics of Isoniazid, Rifampicin, and Pyrazinamide to Whole Plasma, Albumin, and [alpha]-1-Acid Glycoprotein. *Clinical Biochemistry* 1996; 29:175-177.

Chapter 4

Supporting documents

University of Cape Town

Appendices

1. Patient informed consent document
2. Case report forms
3. Ethics approval letter
4. Instructions for authors: Antimicrobial Agents and Chemotherapy

University of Cape Town

Appendix 1

Patient informed consent document

University of Cape Town

PARTICIPANT INFORMATION LEAFLET and INFORMED CONSENTDATE OF FIRST INFORMED CONSENT DISCUSSION: / / (dd/mm/yyyy)**INTRODUCTION:**

You are invited to take part in the research study, entitled:

*The pharmacokinetics of lopinavir in South African HIV-infected volunteers receiving rifampicin with adjusted doses of LOPINAVIR/ritonavir (600/150 mg and 800/200 mg). (APK.DDK study)***RESEARCH INSTITUTION:**

Division of Clinical Pharmacology, K-floor Old Main Building, Groote Schuur Hospital, Observatory.

Tel: 021 406 6008

Fax: 021 448 1989

INVESTIGATORS and STUDY STAFF:

Dr Eric Decloedt	Tel: 021 406 6353	Cell: 083 419 8851
Dr Helen McIlleron	Tel: 021 406 6292	Cell: 084 219 0667
Professor Gary Maartens	Tel: 021 406 6008	
Professor Pete Smith	Tel: 021 406 6289	
Ms Marque Venter (Study Coordinator)	Tel: 021 406 6659	Cell: 074 333 8978

Person conducting informed consent interview:

Contact details:

Tel: Cell:

Before you agree to take part you need to understand why the research is being done and what it will involve. Please read this information leaflet carefully and ask us if anything is not clear or if you need further information. It is important that you have enough time to decide; please don't agree to take part until you are satisfied that you fully understand what will happen, the potential benefits and the risks.

What is the purpose of the research? Tuberculosis (TB) is an infection that commonly causes serious illness, and people with HIV-infection are particularly vulnerable to becoming ill with TB. Some HIV-infected people who get TB will need treatment for both HIV and TB at the same time. Unfortunately, one of the drugs used to treat TB (rifampicin) decreases the amount of some of the antiretroviral drugs in the body, such that the antiretroviral treatment does not work (e.g. lopinavir which is in Kaletra® / Aluvia). Using higher doses of the antiretroviral drugs when they are given with rifampicin might overcome this. This study will measure the amount of lopinavir in the body when the usual doses are given, without and with rifampicin, and when 1.5 and 2 times the usual dose of Kaletra® / Aluvia is given with rifampicin. In this way the research will help to define the right dose of Kaletra® / Aluvia to use in patients who need TB treatment at the same time.

Protocol: APK.DDK

Version 4, Dated 02/06/2009

Participant Initials: _____

Participant Number: _____

Why have you been invited to take part? We are looking for 24 people who are established on antiretroviral treatment including Kaletra®/Aluvia. They must be doing well on their treatment (undetectable viral load), be medically stable, and not taking certain other medications. Furthermore people who have problems with their liver, kidneys or intestine may not take part, and pregnant women or women at risk of becoming pregnant during the study may not take part.

What are your options? You do not have to take part. The decision to take part, or not to take part, is yours. If you decide to take part, you will be asked to sign that you consent to take part. You will be given a copy of this information leaflet to keep. If you decide to take part you are still free to change your mind at any stage; you can withdraw before or during the study. Withdrawing from the study will not affect the standard of medical care that you receive.

What will happen to you if you take part? After signing your consent to take part, the study staff will check your medical records, ask you some questions and they may take a few blood tests to check your liver, to check for hepatitis B infection and to measure your viral load. They will also do an electrocardiogram (ECG) test to check that your heart has normal electrical activity. If these tests are all fine a study time will be scheduled. The study will last 34 days (see schedule on page 6).

Study treatments: During the study period your Kaletra® will be replaced with a study issued product (Aluvia) which contains the same active ingredients as Kaletra®.

- After 1 week of Aluvia in the usual doses, you will be asked to add take the TB drug rifampicin once a day. After a further week, the dose of Aluvia will be increased to 1.5 times the usual dose and a week after that, the dose will be increased to 2 times the usual dose for a week. All the study drugs will then be stopped and you will resume your usual treatment with Kaletra®. Except for Kaletra®, you should continue to take all your medicines as usual throughout the study.
- You will be given a "treatment card" to fill in the times that you take each drug dose during the study period.

Hospital stays: On 4 occasions you will be admitted to the study ward, for measurement of the amount of the study drugs in your blood.

- Each admission will last from a Friday evening to Saturday evening (1 night and one day). If it is difficult for you to get home on the Saturday evening, you can be accommodated until the Sunday morning.
- During the admission you will be provided with food and refreshments, you may not have any other food or drinks. After a meal on the Friday evening you will be allowed only water until about 10 am the following morning, when you will be given a light breakfast.
- On the Saturday morning a cannula (a flexible plastic needle like those used for to give fluids to hospitalized patients) will be placed into one of your arm veins. It will be used for taking blood samples 10

times during the day. The total amount of blood taken on each day will be less than 60 millilitres (12 teaspoons).

Safety monitoring: To monitor the safety of the study treatments we will take regular blood samples to test your liver. You will need to come to the study facility at least once a week (Monday or Tuesday) for 4 weeks and once at the end of the study (Thursday or Friday) for a blood sample to be taken. Furthermore, we will repeat the ECG test at 2 study visits, to ensure that the medication is not affecting your heart.

What are the risks of taking part? You will be taking rifampicin and extra lopinavir/ritonavir (in Aluvia). These extra drugs are of no benefit to you and they might cause bad effects. Moreover, giving the combination of these drugs together might increase the risk of them having bad effects.

- Bad effects of rifampicin include: nausea, vomiting, anorexia, abdominal discomfort, diarrhea, rash, drowsiness and headache. Rare but more serious effects are liver damage confusion, fever, skin reactions, damage to the blood cells and damage to the kidneys. Rifampicin stains urine, tears and other body fluids a reddish-brown to orange. You should not wear contact lenses during the study as they might become stained.
- Bad effects that occur commonly with lopinavir/ritonavir are diarrhoea, nausea and vomiting. Less commonly it causes an unusual fat distribution. Allergic reactions, blood-sugar abnormalities and damage to the liver or pancreas are rare. Recently some patients receiving high doses of lopinavir/ritonavir were noted to have ECG test abnormalities, indicating an effect of lopinavir/ritonavir on the electrical activity of the heart.
- When given together lopinavir/ritonavir and rifampicin may cause an increased risk of liver damage: Studies in volunteers who were given rifampicin for a few days before Kaletra® was added found that there is a high risk of liver damage. We think that this study is safer (patients with HIV-infection and TB have been given the treatments with a low risk of bad consequences) and we think that taking Kaletra® before we start rifampicin is a safer approach. However, liver damage remains a serious concern, so we will monitor your liver regularly.
- The study doctor will tell you if any new information that might affect your decision to take part becomes available.
- The blood-taking process may cause discomfort or bruising, but these will be minimized because experienced health professionals will take the blood samples.
- The amount of lopinavir in your blood may be less than optimal for up to 2 weeks. There is a small possibility this could cause the HIV-virus to become resistant to one of more of the drugs used to fight HIV-infection. However, this risk is very slight as the lopinavir concentrations will only be reduced for a short time and the HIV in your blood is currently well controlled.

What if something goes wrong? If you experience a change in your condition or any problem that might be caused by taking part, please contact the study doctor (or any of the other investigators listed on the first page) WITHOUT DELAY.

If you see any medical professionals outside of the study during the study period, please inform them that you are taking part in the study. Please ask the medical professional to contact us urgently if they are concerned that you might be experiencing problems from the study treatments or if they wish to change any of your medication in any way.

An insurance policy has been taken out to compensate you for reasonable medical expenses related to injury or illness which result from taking part in the study (Internationally accepted guidelines will be used: those laid down by the Association of the British Pharmaceutical Industry [ABPI Guidelines], and Guidelines for Good Clinical Practice in the Conduct of Clinical Trials in Human Participants in South Africa). Compensation will be paid where the injury probably resulted from a drug given to you as part of the study protocol, or any test or procedure you received as part of the study. Payment would be without legal commitment. We would not be bound by these guidelines to pay compensation where the injury resulted from a drug or procedure outside the study protocol, or if the protocol was not followed.

What are the possible benefits of taking part? You will not benefit. The results of the study will benefit future patients who need treatment for TB and HIV at the same time. We will inform your doctor of the lopinavir measurements taken at the first admission. This information might help your doctor to adjust your Kaletra® dose appropriately. However, you are unlikely to have abnormal measurements of lopinavir if you have been taking your treatment as prescribed.

Will your privacy be protected? Yes, as far as is possible. Your medical records will be seen some of the researchers, and by authorized persons checking to see that the research is being carried out correctly. They may also be seen by representatives of the regulatory authority or the responsible ethics committee who may inspect the study records. All these people are obliged to maintain your confidentiality. Your identity will not be disclosed in any publication of the research findings.

What will we use your blood for? Apart from checking that it is safe for you to take part and to monitor your safety during the study, we will use your blood to measure the amounts of the drugs/ medicines in your blood.

What will happen to the blood we take from you? The samples used to measure the drugs will be destroyed 1 year after the study is finished. The samples will not be used for any purposes other than those stated in this document.

Will you be compensated for your travel costs, time and inconvenience? Yes, you will be given an amount of R50 for short visits and R310 for admissions to compensate you for your time, the inconvenience and travel costs.

What will happen with the results of the research? The results of the study will be published so that they are available to the medical profession throughout the world. No patients will be identified individually.

What organizations are supporting the study? The study is financed by the European and Developing Countries Clinical Trials Partnership (EDCTP) and the South African Department of Health. It is supported and organized by the University of Cape Town.

What if you have complaints about the study? If you want any information regarding your rights as a research participant, or have complaints regarding this research, you may contact Prof. Marc Blockman, the **Chairperson of the Research Ethics Committee** at the University of Cape Town (021 406 6492). After you have consulted your doctor or the ethics committee and they have not provided you with answers to your satisfaction, you should write to: The Registrar, South African Medicines Control Council (MCC), Department of Health, Private Bag X 828, PRETORIA 0001.

University of Cape Town

Study Schedule:

DATE	DAY	EVENT
-30 to -6	-1/-6 [Friday/Saturday]	Study medicine for the next week will be dispensed.
-5 to 0	[Sunday to Friday]	Take usual doses of Aluvia (2 tablets 12 hourly) in place of Kaletra® (3 tablets 12 hourly). Continue with your other medicines (except Kaletra®) as usual.
0	[Friday]	Arrive for admission to ward at 19h00 / 7 pm (J51, Old Main Building, Groote Schuur). Bring your remaining study medicine and your other medicines with you.
1	[Saturday]	1 st blood sampling day in the ward. You may go home at about 20h30 / 8.30 pm. Study medicines for the next week will be dispensed.
2 to 7	[Sunday to Friday]	Take week 2 of study medicines (2 tablets of Aluvia 12 hourly and 1 tablet of rifampicin daily). Continue with your other medicines (except Kaletra®) as usual.
3 or 4	[Monday or Tuesday]	Arrive at J51 at about 8h00 / 8 am for check up and safety blood samples.
7	[Friday]	Arrive for admission to ward at 19h00 / 7 pm (J51, Old Main Building, Groote Schuur). Bring your remaining study medicine and your other medicines with you.
8	[Saturday]	2 nd blood sampling day in the ward. You may go home at about 20h30 / 8.30 pm. Study medicines for the next week will be dispensed.
9 to 14	[Sunday to Friday]	Take week 3 of study medicines (3 tablets of Aluvia 12 hourly and 1 tablet of rifampicin daily). Continue with your other medicines (except Kaletra®) as usual.
10 or 11	[Monday or Tuesday]	Arrive at J51 at about 8h00 / 8 am for check up and safety blood samples.
14	[Friday]	Arrive for admission to ward at 19h00 / 7 pm (J51, Old Main Building, Groote Schuur). Bring your remaining study medicine and your other medicines with you.
15	[Saturday]	3 rd blood sampling day in the ward. You may go home at about 20h30 / 8.30 pm. Study medicines for the next week will be dispensed.
16 to 21	[Sunday to Friday]	Take week 4 of study medicines (4 tablets of Aluvia 12 hourly and 1 tablet of rifampicin daily). Continue with your other medicines (except Kaletra®) as usual.
17 or 18	[Monday or Tuesday]	Arrive at J51 at about 8h00 / 8 am for check up and safety blood samples.
21	[Friday]	Arrive for admission to ward at 19h00 / 7 pm (J51, Old Main Building, Groote Schuur). Bring your remaining study medicine and your other medicines with you.
22	[Saturday]	Last blood sampling day in the ward. You may go home at about 20h30 / 8.30 pm. The study drugs are now finished and you need to resume your usual doses of Kaletra® after the last blood sample at about 20h00 / 8pm.
24 or 25	[Monday or Tuesday]	Arrive at J51 at about 8h00 / 8 am for check up and safety blood samples.
28	[Friday]	Arrive at J51 at about 8h00 / 8 am for last study check up and safety blood samples.

Protocol: APR.DDK

Version 4, Dated 02/05/2009

Participant Number: _____

Participant Initials: _____

INFORMED CONSENT FORM

.....(STUDY DOCTOR/DESIGNATED STUDY TEAM MEMBER) has provided me with a copy of the Participant Information Leaflet regarding the study, "The pharmacokinetics of lopinavir in South African HIV-infected volunteers receiving rifampicin with adjusted doses of LOPINAVIR/ritonavir (600/150 mg and 800/200 mg)."

and has fully explained to me the nature, risks, benefits and purpose of the study.

- I have been given the opportunity to ask any questions concerning the research study procedures, the potential benefits and risks.
- It has been explained to me that I am free to withdraw from the study at any time, without any disadvantage to future care.
- I understand everything that has been explained to me and I consent to take part in this research study.

PARTICIPANT:

<hr/> <i>Print name</i>	<hr/> <i>Signature / Thumbprint</i>	<hr/> <i>Date</i>
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STUDY DOCTOR / DESIGNATED STUDY TEAM MEMBER:

<hr/> <i>Print name</i>	<hr/> <i>Signature</i>	<hr/> <i>Date</i>
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TRANSLATOR / PERSON EXPLAINING INFORMED CONSENT:

<hr/> <i>Print name</i>	<hr/> <i>Signature</i>	<hr/> <i>Date</i>
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WITNESS:

<hr/> <i>Print name</i>	<hr/> <i>Signature</i>	<hr/> <i>Date</i>
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Appendix 2

Case report forms

University of Cape Town

Study Volunteer Screening #		INITIALS			
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ELIGIBILITY ASSESSMENT

Inclusion criteria:		
1	Has informed consent been signed by volunteer prior to study entry?	yes no
2	Has the volunteer been documented to be HIV-infected by positive HIV-antibody test?	yes no
3	Is the volunteer older than 18 years?	yes no
4	Has the volunteer been established on an antiretroviral regimen comprising LPV 400 mg/RTV 100 mg and 2 NRTIs for more than 6 months?	yes no
5	Has the volunteer an undetectable viral load of less than 50 viral copies/mL that will be less than 12 weeks old when the study starts?	yes no
6	Has the volunteer an ALT value of less than 1.5 X the upper limit of normal less than 1 week old?	yes no
7	Were hepatitis studies done less than 12 weeks ago and is the volunteer hepatitis B virus surface antigen negative and HCV Ab negative?	yes no
8	In your opinion, is the patient medically stable?	yes no
Exclusion criteria:		
1	Did the volunteer previously fail to attain or maintain virological suppression on a protease inhibitor-containing regimen?	yes no
2	Is the volunteer known to have chronic renal, hepatic or GIT disease that may interfere with the pharmacokinetics of the drugs studied?	yes no
3	Does the volunteer have a <ul style="list-style-type: none"> fasting cholesterol > 7.77 mmol/L? fasting triglyceride > 8.49 mmol/L? casual plasma glucose measurements > 11.1 mmol/L or fasting plasma glucose ≥ 7.0 mmol/L done at baseline or 6 monthly checks? 	yes no
4	Does the volunteer consume in excess of 2 units/day or 14 units/week alcohol?	yes no
5	Has tuberculosis (TB) been excluded?	yes no
6	Is the volunteer receiving drugs other than the study medication known to potently induce or inhibit CYP3A4 or alter the PK of LPV; at the discretion of the PI?	yes no
7	Is the volunteer receiving drugs (e.g. Oral contraceptive) other than the study drugs whose PK is known to be altered (with potentially important consequences) by Kaletra®?	yes no
8	Is the volunteer known or suspected to be pregnant?	yes no
9	If the patient is female or of child-bearing potential, is she using a recognized form of contraception?	yes no

PARTICIPANT SCREENING AND ENROLMENT INFORMATION

APK.DDK-study

Study Volunteer
Screening #

INITIALS

SCREENING VISIT DATE: / / 20 (DD/MM/YYYY)

Informed consent

Consent signed prior to any study-related procedures being performed?

Yes / No

Date signed:

..... / / 20 (DD/MM/YYYY)

Study Volunteer Demographics

Date of birth:

..... / / 19 (DD/MM/YYYY)

Sex

: Male / Female

Race

: Black / White / Coloured / Asian / other:

General Information:Contraception:

If female, is she using contraception:

Yes / No

If yes: what type of contraception is being used?

Alcohol:

Do you use alcohol?

Yes / No

If yes:

Type of alcohol: Wine/beer/spirits/other:

Units per week?

Do you binge drink?

Yes / No

If yes, how many units per episode:

Smoking:

Smoking classification:

Never smoked

Ex-smoker

Smoker

Drug abuse:

Do you use recreational drugs?

Yes / No

If yes, what do you use/how often/last use?

Clinical trials:

Have you taken part in any other clinical trials during the last 2 months?

Yes / No

If yes, provide details of the trial as well as the study drug used:

NOTES:

PARTICIPANT SCREENING AND ENROLMENT INFORMATION

APK.DDK-study

Study Volunteer
Screening #

INITIALS

General medical historyAllergies

Do you have any allergies to medicines?

Yes / No

If yes, specify:

Contraindications to rifampicin:

Do you have porphyria?

Yes / No

Have you used rifampicin (TB treatment) before?

Yes / No

If yes:

Did you develop a skin reaction or a rash accompanied by a fever? :

Yes / No

Did you develop liver toxicity or jaundice?

Yes / No

Elaborate:

Adverse events due to lopinavir/ritonavir:

Did you experience any adverse events or side-effects on lopinavir/ritonavir?:

Yes / No

Elaborate:

NOTES

PARTICIPANT SCREENING AND ENROLMENT INFORMATION

APK.DDK-study

Study Volunteer Screening #		INITIALS	
--------------------------------	--	----------	--

Medical history:

Have you ever suffered from problems with your:			
System			Description
Head & Neck	Yes	No	
Eyes	Yes	No	
Ears, nose, throat/mouth	Yes	No	
Thyroid	Yes	No	
Heart	Yes	No	
Lungs	Yes	No	
Gastrointestinal	Yes	No	
Liver	Yes	No	
Urogenital tract	Yes	No	
Musculoskeletal	Yes	No	
Central nervous system	Yes	No	
Blood	Yes	No	
Skin	Yes	No	

HAART Regimen

	Date started	Date stopped	REGIMEN						Reason stopped	
				Dose		Dose		Dose		
1			d4T		AZT		EFV/NVP		Failure	Toxicity
2			d4T		AZT		EFV/NVP		Failure	Toxicity
3									Failure	Toxicity
4									Failure	Toxicity
5									Failure	Toxicity

WHO

Staging when starting HAART:

NOTES:

PARTICIPANT SCREENING AND ENROLMENT INFORMATION

APK.DDK-study

Study Volunteer
Screening #

INITIALS

Excluding TB

Did you loose weight in the last 4 weeks?

Yes / No

If yes, what was the weight before? ____ kg

OR

dress/pant size before? ____

What is your weight now? ____ kg

OR

dress/pant size now? ____

Have you been coughing for more than 2 weeks?

Yes / No

Have you been having fever for more than 2 weeks?

Yes / No

Have you been having nightsweats for more than 2 weeks?:

Yes / No

Medication

What medication did you take in the last 3 months?	Why did you take it?	When did you start taking it?	Are you still taking it?		If no, when did you stop taking it?	Dose used
			Yes	No		
			Yes	No		
			Yes	No		
			Yes	No		
			Yes	No		
			Yes	No		
			Yes	No		
			Yes	No		
			Yes	No		
			Yes	No		

Surgical history:

Have you had any surgical procedures in the last year?

Yes / No

If yes, provide details:

PARTICIPANT SCREENING AND ENROLMENT INFORMATION

APK.DDK-study

Study Volunteer
Screening #

INITIALS

Physical examination

Body weight	Height	Oral temperature	Supine BP	Supine pulse
kg	cm	°C	mmHg	bpm

Body system	Not done	Normal	Abnormal (specify)
General appearance			
Head and neck			
Eyes, ears, nose, throat, mouth			
Cardiovascular			
Pulmonary			
Abdomen			
Urogenital			
Musculoskeletal			
Neurological			
Skin			
Other			

Comments

University of Cape Town

Special investigations:

CXR done?

Yes / No

If yes,

Date

/ / 20 (DD / MM / YY)

Done by

Reported by

Suggestive of PTB

Yes / No

If yes, provide details:

.....

.....

PARTICIPANT SCREENING AND ENROLMENT INFORMATION

APK.DDK-study

Study Volunteer
Screening #

INITIALS

Laboratory Investigations:

Blood test	Done	Date	Result attached	Not done	Repeat today
HIV ELISA					
<i>Baseline tests done on starting lopinavir-based HAART</i>					
Viral load					X
CD4+ cell count					X
ALT					X
FBC					X
Random glucose					X
Fasting glucose					X
Triglycerides					X
Cholesterol					X
<i>Tests done on last 6 monthly routine visit</i>					
Viral load ¹					
CD4+ cell count					
ALT ²					
FBC					
Random glucose					
Fasting glucose					
Triglycerides					
Cholesterol					
HBVsAg ¹					
HCV Ab ¹					
¹ Repeat test if result is more than 12 weeks old					
² Repeat test if result is more than 1 week old					

PATIENT INCLUDED IN STUDY:

YES / NO

IF YES: PARTICIPANT ID / Enrolment #

D _____

IF NO:

REASON _____

FORM COMPLETED BY:

NAME: _____

SIGNATURE _____

Study Role _____

DATE: _____

PARTICIPANT
STUDY ID #

D

INITIALS

ADMISSION DATE: / / 2009 17/07/2009

TIME: h

History

Adherence:

Pill count done

: Yes / No

Treatment card checked

: Yes / No

Check list for new treatment of A.E's completed : Yes / No

Vital signs

Body weight	Temperature	Supine BP	Supine pulse
kg	°C	/ mmHg	bpm

Clinical notes

University of Cape Town

Investigator signature:Date:

Time of Friday evening Aluvia® dose (pre-PK sampling dose): h

Time of dose observed ☐ / obtained from volunteer history ☐Blood samples to be taken from volunteer after cannulating on PK sampling day:

Blood test	Done	Blood test	Done
<i>Baseline bloods</i>		<i>Safety bloods</i>	
Creatinine		ALT	
CRP		Total bilirubin	
Albumin		FBC	

PARTICIPANT
STUDY ID #

D

INITIALS

DATE: ... / ... / 2009 (DD / MM / YYYY) TIME: ... h ...

History

Check list for new treatment and A.E's completed? : Yes / No

Vital signs

Temperature	Supine BP	Supine pulse
_____ °C	____ / ____ mmHg	_____ bpm

Clinical notes

University of Cape Town

Laboratory investigations

Blood test	Done
Safety bloods	
ALT	
Total bilirubin	

Investigator signature:Date:

ADVERSE EVENT RECORD

APK-DDK-STUDY

PARTICIPANT STUDY ID #	D		INITIALS	
---------------------------	---	--	----------	--

ADVERSE EVENT RECORD					
Date	Visit	Adverse event?	Type of adverse event	Adverse event form completed?	
..... / / 2009	Screening	Yes / No	Baseline	Yes / No	
..... / / 2009	PK	Yes / No	Now adverse event	Yes / No	
	Safety		Follow up on previous adverse event		
	Additional safety				
..... / / 2009	PK	Yes / No	New adverse event	Yes / No	
	Safety		Follow up on previous adverse event		
	Additional safety				
..... / / 2009	PK	Yes / No	Now adverse event	Yes / No	
	Safety		Follow up on previous adverse event		
	Additional safety				
..... / / 2009	PK	Yes / No	New adverse event	Yes / No	
	Safety		Follow up on previous adverse event		
	Additional safety				
..... / / 2009	PK	Yes / No	Now adverse event	Yes / No	
	Safety		Follow up on previous adverse event		
	Additional safety				
..... / / 2009	PK	Yes / No	Now adverse event	Yes / No	
	Safety		Follow up on previous adverse event		
	Additional safety				
..... / / 2009	PK	Yes / No	Now adverse event	Yes / No	
	Safety		Follow up on previous adverse event		
	Additional safety				
..... / / 2009	PK	Yes / No	Now adverse event	Yes / No	
	Safety		Follow up on previous adverse event		
	Additional safety				
..... / / 2009	PK	Yes / No	Now adverse event	Yes / No	
	Safety		Follow up on previous adverse event		
	Additional safety				
..... / / 2009	PK	Yes / No	Now adverse event	Yes / No	
	Safety		Follow up on previous adverse event		
	Additional safety				

ADVERSE EVENT RECORD

APK.DDK-STUDY

PARTICIPANT
STUDY ID #

D

INITIALS

ADVERSE EVENT RECORD				
Date	Visit	Adverse event?	Type of adverse event	Adverse event form completed?
..... / / 2009	PK	Yes / No	New adverse event	Yes / No
	Safety		Follow up on previous adverse event	
	Additional safety			
..... / / 2009	PK	Yes / No	New adverse event	Yes / No
	Safety		Follow up on previous adverse event	
	Additional safety			
..... / / 2009	PK	Yes / No	New adverse event	Yes / No
	Safety		Follow up on previous adverse event	
	Additional safety			
..... / / 2009	PK	Yes / No	New adverse event	Yes / No
	Safety		Follow up on previous adverse event	
	Additional safety			
..... / / 2009	PK	Yes / No	New adverse event	Yes / No
	Safety		Follow up on previous adverse event	
	Additional safety			
..... / / 2009	PK	Yes / No	New adverse event	Yes / No
	Safety		Follow up on previous adverse event	
	Additional safety			
..... / / 2009	PK	Yes / No	New adverse event	Yes / No
	Safety		Follow up on previous adverse event	
	Additional safety			
..... / / 2009	PK	Yes / No	New adverse event	Yes / No
	Safety		Follow up on previous adverse event	
	Additional safety			
..... / / 2009	PK	Yes / No	New adverse event	Yes / No
	Safety		Follow up on previous adverse event	
	Additional safety			
..... / / 2009	PK	Yes / No	New adverse event	Yes / No
	Safety		Follow up on previous adverse event	
	Additional safety			

ADVERSE EVENT ASSESSMENT FORM

PARTICIPANT'S STUDY ID

D

DATE

dd

mm

2009

Name of person completing form _____

Section 1

(A) Does the participant report any problems with taking the medication?

☐ Y☐ N

If YES, describe: _____

(B) Does the patient associate the problem/s with particular medicine/s?

☐ Y☐ N

If YES, describe: _____

Section 2

Do any findings on history or examination meet the definition of an adverse event (AE)?

☐ Y☐ N

An AE is any untoward medical occurrence which does not necessarily have a causal relationship with this trial treatment. It can be any unfavourable and unintended sign (incl an abnormal laboratory finding), symptom or disease temporally associated with the use of the study drug, whether or not related.

If YES, please describe on page 2. Grade using the DAIDS system in the study file.

If AE is grade 3 or 4, the participant must be withdrawn from the study and the DSMB informed. If the AE is serious (results in death, is life threatening, requires hospitalisation or prolongation of a hospital admission, is medically important/necessitates change in ARV regimen), contact PI (thus REC, MCC) asap; Fill out SAE form

Section 3

Are any laboratory results abnormal?

☐ Y☐ N

IF YES, please record on page 3. Use the DAIDS grading system in the study file

If AE is grade 3 or 4, the participant must be withdrawn from the study and the DSMB informed. If the AE is serious (results in death, is life threatening, requires hospitalisation or prolongation of a hospital admission, is medically important/necessitates change in ARV regimen), contact PI (thus REC, MCC) asap; Fill out SAE form

Notes:

ADVERSE EVENT ASSESSMENT FORM - page 2

PARTICIPANT'S STUDY ID

D

DATE

dd

mm

2009

AE 1 description

(use DAIDS categories
if applicable)

when did it start?

d d m m y y

duration

/ ongoing

please grade the event - use DAIDS system in study file

1

2

3

4

does it meet the definition of serious?

Y

N

is it associated with any particular medicine/s?

YES

probably

possibly

NO

which drug/s?

Action

☐ none☐ increased observation☐ hospitalization☐ medication (as below)

Treatment plan

no change ☐drugs started ☐drugs stopped ☐

reason:

drug:

AE 2 description

(use DAIDS categories
if applicable)

when did it start?

d d m m y y

duration

/ ongoing

please grade the event - use DAIDS system in study file

1

2

3

4

does it meet the definition of serious?

Y

N

is it associated with any particular medicine/s?

YES

probably

possibly

NO

which drug/s?

Action

☐ none☐ increased observation☐ hospitalization☐ medication (as below)

Treatment plan

no change ☐drugs started ☐drugs stopped ☐

reason

drug:

Please photocopy more sheets if more AE's

Notes:

ADVERSE EVENT ASSESSMENT FORM - page 3

PARTICIPANT'S STUDY ID

D

DATE

dd

mm

2009

Section 3

Please report any abnormal laboratory results

Please grade the AE using the DAIDS system in the study file

Please specify the date the test was taken

TEST:	RESULT:	GRADE:	DATE of TEST:
1 <input style="width: 150px;" type="text"/>	<input style="width: 130px;" type="text"/>	1 2 3 4	<input style="width: 20px;" type="text"/> d <input style="width: 20px;" type="text"/> d <input style="width: 20px;" type="text"/> m <input style="width: 20px;" type="text"/> m <input style="width: 20px;" type="text"/> y <input style="width: 20px;" type="text"/> y
relation to drug: <input type="checkbox"/> YES	<input type="checkbox"/> probably <input type="checkbox"/> possibly	<input type="checkbox"/> NO	which drug/s: <input style="width: 150px;" type="text"/>
2 <input style="width: 150px;" type="text"/>	<input style="width: 130px;" type="text"/>	1 2 3 4	<input style="width: 20px;" type="text"/> d <input style="width: 20px;" type="text"/> d <input style="width: 20px;" type="text"/> m <input style="width: 20px;" type="text"/> m <input style="width: 20px;" type="text"/> y <input style="width: 20px;" type="text"/> y
relation to drug: <input type="checkbox"/> YES	<input type="checkbox"/> probably <input type="checkbox"/> possibly	<input type="checkbox"/> NO	which drug/s: <input style="width: 150px;" type="text"/>
3 <input style="width: 150px;" type="text"/>	<input style="width: 130px;" type="text"/>	1 2 3 4	<input style="width: 20px;" type="text"/> d <input style="width: 20px;" type="text"/> d <input style="width: 20px;" type="text"/> m <input style="width: 20px;" type="text"/> m <input style="width: 20px;" type="text"/> y <input style="width: 20px;" type="text"/> y
relation to drug: <input type="checkbox"/> YES	<input type="checkbox"/> probably <input type="checkbox"/> possibly	<input type="checkbox"/> NO	which drug/s: <input style="width: 150px;" type="text"/>
4 <input style="width: 150px;" type="text"/>	<input style="width: 130px;" type="text"/>	1 2 3 4	<input style="width: 20px;" type="text"/> d <input style="width: 20px;" type="text"/> d <input style="width: 20px;" type="text"/> m <input style="width: 20px;" type="text"/> m <input style="width: 20px;" type="text"/> y <input style="width: 20px;" type="text"/> y
relation to drug: <input type="checkbox"/> YES	<input type="checkbox"/> probably <input type="checkbox"/> possibly	<input type="checkbox"/> NO	which drug/s: <input style="width: 150px;" type="text"/>
5 <input style="width: 150px;" type="text"/>	<input style="width: 130px;" type="text"/>	1 2 3 4	<input style="width: 20px;" type="text"/> d <input style="width: 20px;" type="text"/> d <input style="width: 20px;" type="text"/> m <input style="width: 20px;" type="text"/> m <input style="width: 20px;" type="text"/> y <input style="width: 20px;" type="text"/> y
relation to drug: <input type="checkbox"/> YES	<input type="checkbox"/> probably <input type="checkbox"/> possibly	<input type="checkbox"/> NO	which drug/s: <input style="width: 150px;" type="text"/>
6 <input style="width: 150px;" type="text"/>	<input style="width: 130px;" type="text"/>	1 2 3 4	<input style="width: 20px;" type="text"/> d <input style="width: 20px;" type="text"/> d <input style="width: 20px;" type="text"/> m <input style="width: 20px;" type="text"/> m <input style="width: 20px;" type="text"/> y <input style="width: 20px;" type="text"/> y
relation to drug: <input type="checkbox"/> YES	<input type="checkbox"/> probably <input type="checkbox"/> possibly	<input type="checkbox"/> NO	which drug/s: <input style="width: 150px;" type="text"/>
7 <input style="width: 150px;" type="text"/>	<input style="width: 130px;" type="text"/>	1 2 3 4	<input style="width: 20px;" type="text"/> d <input style="width: 20px;" type="text"/> d <input style="width: 20px;" type="text"/> m <input style="width: 20px;" type="text"/> m <input style="width: 20px;" type="text"/> y <input style="width: 20px;" type="text"/> y
relation to drug: <input type="checkbox"/> YES	<input type="checkbox"/> probably <input type="checkbox"/> possibly	<input type="checkbox"/> NO	which drug/s: <input style="width: 150px;" type="text"/>

ACTIONS: ☐ none ☐ increased observation ☐ hospitalization ☐ medication (as below)
TREATMENT PLAN:no change ☐drugs started ☐drugs stopped ☐

reason

drug:

Notes:

PK SAMPLING VISIT ____

Date of PK ____ sampling: du-____-09

Study title: The pharmacokinetics of lopinavir in South African HIV-infected volunteers receiving rifampicin with adjusted doses of LOPINAVIR/ritonavir (600/150 mg and 800/200 mg)

DRUG DOSING DETAILS

Aluvia® (400/100mg) ☐ tablets administered Time of dose: : : :

Observed by: _____

Signature: _____

SPECIMEN DETAILS

Sample ID	Sampling time (after drug administration)			Comments
	Time (H)	Scheduled (hhmm)	Actual (hhmm)	
D____-____-01	0.5	<input type="text"/>	<input type="text"/>	
D____-____-02	1.5	<input type="text"/>	<input type="text"/>	
D____-____-03	2	<input type="text"/>	<input type="text"/>	
D____-____-04	2.5	<input type="text"/>	<input type="text"/>	
D____-____-05	3	<input type="text"/>	<input type="text"/>	
D____-____-06	4	<input type="text"/>	<input type="text"/>	
D____-____-07	5	<input type="text"/>	<input type="text"/>	
D____-____-08	6	<input type="text"/>	<input type="text"/>	
D____-____-09	8	<input type="text"/>	<input type="text"/>	
D____-____-10	12	<input type="text"/>	<input type="text"/>	

NAME AND SIGNATURE

Name		Signature
Person responsible for PK sampling		

NOTES:

This form should be filled in duplicate

Appendix 3

Ethics approval letter

University of Cape Town

Appendix 4

Instructions for authors:

Antimicrobial Agents and Chemotherapy

University of Cape Town

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY

2011 INSTRUCTIONS TO AUTHORS*

SCOPE

Antimicrobial Agents and Chemotherapy (AAC) is an interdisciplinary journal devoted to the dissemination of knowledge relating to all aspects of antimicrobial and antiparasitic agents and chemotherapy. Within the circumscriptions set forth below, any report involving studies of or with antimicrobial, antiviral (including antiretroviral), antifungal, or antiparasitic agents as these relate to human disease is within the purview of AAC. Studies involving animal models, pharmacological characterization, and clinical trials are appropriate for consideration.

ASM publishes a number of different journals covering various aspects of the field of microbiology. Each journal has a prescribed scope that must be considered in determining the most appropriate journal for each manuscript. The following guidelines may be of assistance.

(i) Papers which describe the use of antimicrobial agents as tools for elucidating the basic biological processes of bacteria are considered more appropriate for the *Journal of Bacteriology*.

(ii) Manuscripts that (a) describe the use of antimicrobial or antiparasitic agents as tools in the isolation, identification, or epidemiology of microorganisms associated with disease; (b) are concerned with quality control procedures for diffusion, elution, or dilution tests for determining susceptibilities to antimicrobial agents in clinical laboratories; and (c) deal with applications of commercially prepared tests or kits to assays performed in clinical laboratories to measure the activities of established antimicrobial agents or their concentrations in body fluids are considered more appropriate for the *Journal of Clinical Microbiology*. Manuscripts concerned with the development or modification of assay methods (e.g., plasma antimicrobial concentrations and high-throughput screening techniques, etc.) and validation of their sensitivity and specificity with a sufficiently large number of determinations or compounds are considered appropriate for AAC.

(iii) Manuscripts describing new or novel methods or improvements in media and culture conditions will not be considered for publication in AAC unless these methods are applied to the study of problems related to the production or activity of antimicrobial agents. Such manuscripts are more appropriate for *Applied and Environmental Microbiology* or the *Journal of Clinical Microbiology*.

(iv) Manuscripts dealing with properties of unpurified natural products, with entities that are primarily

antitumor agents, or with immunomodulatory agents that are not antimicrobial agents are not appropriate for AAC.

(v) Manuscripts dealing with novel small molecular antimicrobials must provide at least some data showing that the proposed new agents or scaffolds have the potential to become therapeutic agents. Appropriate demonstrations will vary but generally should be some combination of data on physical properties (solubility, protein binding, log *P* [logarithm of the ratio of the concentrations of un-ionized solute in solvents]), pharmacological properties (Caco2 predictions of bioavailability, pharmacokinetics in an animal species), or tolerability (mammalian cell toxicity, likelihood of hepatic metabolism, potential for receptor interactions, potential for human ERG liability). Initial presentations of compounds are not expected to address all these areas but rather to show an appropriate initial subset. For example, the first publication of a novel compound or compound series might address selected physical properties plus mammalian cell toxicity. Subsequent publications are expected to add progressively to the proof of the agent's therapeutic potential.

(vi) Biochemical analyses for β -lactamases that determine kinetic parameters (e.g., K_m , k_{cat}) must be performed on purified enzyme preparations. The enzyme must be in its native form, without any leader sequences or fusions used for purification (e.g., His tag). The determination of relative rates of hydrolysis may be performed on crude extracts.

(vii) Authors of papers describing enzymological studies should review the standards of the STREND A Commission for information required for adequate description of experimental conditions and for reporting enzyme activity data (<http://www.beilstein-institut.de/en/projekte/strenda/guidelines/>).

(viii) A manuscript limited to the nucleic acid sequence of a gene encoding an antibiotic target, receptor, or resistance mechanism may be submitted as a short-form paper (see "Short-Form Papers") or a New-Data Letter to the Editor (see "Letters to the Editor"), depending on its length. Formatting instructions for nucleic acid sequences are given below (see "Presentation of Nucleic Acid Sequences"). Repetition of sequences already in a database should be avoided.

Questions about these guidelines may be directed to the editor in chief of the journal being considered.

If transfer to another ASM journal is recommended by an editor, the corresponding author will be contacted.

Note that a manuscript rejected by one ASM journal on scientific grounds or on the basis of its general suitability for publication is considered rejected by all other ASM journals.

*Instructions to Authors are published annually in the January issue. A separate html version, which is updated throughout the year, is at <http://aac.asm.org/misc/fora.dtl>.

EDITORIAL POLICY

Use of Microbiological Information

The Council Policy Committee (CPC) of the American Society for Microbiology affirms the long-standing position of the Society that microbiologists will work for the proper and beneficent application of science and will call to the attention of the public or the appropriate authorities misuses of microbiology or of information derived from microbiology. ASM members are obligated to discourage any use of microbiology contrary to the welfare of humankind, including the use of microbes as biological weapons. Bioterrorism violates the fundamental principles expressed in the Code of Ethics of the Society and is abhorrent to ASM and its members.

ASM recognizes that there are valid concerns regarding the publication of information in scientific journals that could be put to inappropriate use as described in the CPC resolution mentioned above. Members of the ASM Publications Board will evaluate the rare manuscript that might raise such issues during the review process. However, as indicated elsewhere in these Instructions, research articles must contain sufficient detail, and material/information must be made available, to permit the work to be repeated by others. Supply of materials should be in accordance with laws and regulations governing the shipment, transfer, possession, and use of biological materials and must be for legitimate, bona fide research needs. Links to, and information regarding, these laws and regulations can be found at <http://www.asm.org/> under the Public Policy tab. We ask that authors pay particular attention to the NSAR Select Agent/Toxin list on the CDC website <http://www.selectagents.gov/index.html> and the NSABB criteria for identifying dual use research of concern in the report "Proposed Framework for the Oversight of Dual Use Life Sciences Research: Strategies for Minimizing the Potential Misuse of Research Information" on the Office of Biotechnology Activities website http://oba.od.nih.gov/biosecurity/pdf/Framework%20for%20transmittal%200807_Sept07.pdf (p. 17–22).

Ethical Guidelines

ASM requirements for submitted manuscripts are consistent with the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, as last updated by the International Committee of Medical Journal Editors in April 2010 (<http://www.icmje.org>).

Authors are expected to adhere to the highest ethical standards. The following sections of these Instructions include detailed information about ASM's ethical standards. Failure to comply with the policies described in these Instructions may result in a letter of reprimand, a suspension of publishing privileges in ASM journals, and/or notification of the authors' institutions. Authors employed by companies whose policies do not permit them to comply with ASM policies may be sanctioned as individuals and/or ASM may refuse to consider manuscripts having

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ORGANIZATION AND FORMAT

Editorial Style

The editorial style of ASM journals conforms to the *ASM Style Manual for Journals* (American Society for Microbiology, 2011, in-house document) and *How To Write and Publish a Scientific Paper*, 6th ed. (Greenwood Press, Westport, CT, 2006), as interpreted and modified by the editors and the ASM Journals Department.

The editors and the Journals Department reserve the privilege of editing manuscripts to conform with the stylistic conventions set forth in the aforesaid publications and in these Instructions.

On receipt at ASM, an accepted manuscript undergoes an automated preediting, cleanup, and tagging process specific to the particular article type. To optimize this process, manuscripts must be supplied in the correct format and with the appropriate sections and headings.

Type every portion of the manuscript double-spaced (a minimum of 6 mm between lines), including figure legends, table footnotes, and references, and number all pages in sequence, including the abstract, figure legends, and tables. Place the last two items after the References section. Manuscript pages must have line numbers; manuscripts without line numbers may be editorially rejected by the editor, with a suggestion of resubmission after line numbers are added. The font size should be no smaller than 12 points. It is recommended that the following sets of characters be easily distinguishable in the manuscript: the numeral zero (0) and the letter "oh" (O); the numeral one (1), the letter "el" (l), and the letter "eye" (I); and a multiplication sign (×) and the letter "ex" (x). Do not create symbols as graphics or use special fonts that are external to your word processing program; use the "insert symbol" function. Set the page size to 8½ by 11 inches (ca. 21.6 by 28 cm). Italicize any words that should appear in italics, and indicate paragraph lead-ins in boldface type.

Authors who are unsure of proper English usage should have their manuscripts checked by someone proficient in the English language.

Manuscripts may be editorially rejected, without review, on the basis of poor English or lack of conformity to the standards set forth in these Instructions.

Full-Length Papers

Full-length papers should include the elements described in this section.

Title, running title, and byline. Each manuscript should present the results of an independent, cohesive study; thus, numbered series titles are not permitted.

Exercise care in composing a title. Avoid the main title/subtitle arrangement, complete sentences, and unnecessary articles. On the title page, include the title, the running title (not to exceed 54 characters and spaces), the name of each author, the address(es) of the institution(s) at which the work was performed, each author's affiliation, and a footnote indicating the present address of any author no longer at the institution where the work was performed. Place an asterisk after the name of the author to whom inquiries regarding the paper should be directed (see "Correspondent footnote," below).

Study group in byline. A study group, surveillance team, working group, consortium, or the like (e.g., the Active Bacterial Core Surveillance Team) may be listed as a coauthor in the byline if its contributing members satisfy the requirements for authorship and accountability as described in these Instructions. The names (and institutional affiliations if desired) of the contributing members may be given in a footnote keyed to the study group name in the byline or as a separate paragraph in Acknowledgments.

If the contributing members of the group associated with the work do not fulfill the criteria of substantial contribution to and responsibility for the paper, the group may not be listed in the author byline. Instead, it and the names of its contributing members may be listed in the Acknowledgments section.

Correspondent footnote. The complete mailing address, a single telephone number, a single fax number, and a single e-mail address for the corresponding author should be included on the title page of the manuscript. This information will be published in the article as a footnote to facilitate communication, and the e-mail address will be used to notify the corresponding author of the availability of proofs and, later, of the PDF file of the published article. No more than two authors may be designated corresponding authors.

Abstract. Limit the abstract to 250 words or fewer and concisely summarize the basic content of the paper without presenting extensive experimental details. Avoid abbreviations and references, and do not include diagrams. When it is essential to include a reference, use the same format as shown for the References section but omit the article title. Conclude the abstract with a summary statement. Because the abstract will be published separately by abstracting services, it must be complete and understandable without reference to the text.

Introduction. The introduction should supply sufficient background information to allow the reader to understand and evaluate the results of the present study without referring to previous publications on the topic. The introduction should also provide the hypothesis that was addressed or the rationale for the study. References

should be chosen carefully to provide the most salient background rather than an exhaustive review of the topic.

Case Report. The Case Report section, placed after the introduction and before Materials and Methods, is optional and gives relevant clinical information about one or more patients.

Materials and Methods. The Materials and Methods section should include sufficient technical information to allow the experiments to be repeated. When centrifugation conditions are critical, give enough information to enable another investigator to repeat the procedure: make of centrifuge, model of rotor, temperature, time at maximum speed, and centrifugal force ($\times g$ rather than revolutions per minute). For commonly used materials and methods (e.g., media and protein concentration determinations), a simple reference is sufficient. If several alternative methods are commonly used, it is helpful to identify the method briefly as well as to cite the reference. For example, it is preferable to state "cells were broken by ultrasonic treatment as previously described (9)" rather than "cells were broken as previously described (9)." This allows the reader to assess the method without constant reference to previous publications. Describe new methods completely, and give sources of unusual chemicals, equipment, or microbial strains. When large numbers of microbial strains or mutants are used in a study, include tables identifying the immediate sources (i.e., sources from whom the strains were obtained) and properties of the strains, mutants, bacteriophages, and plasmids, etc.

A method or strain, etc., used in only one of several experiments reported in the paper may be described in the Results section or very briefly (one or two sentences) in a table footnote or figure legend. It is expected that the sources from whom the strains were obtained will be identified.

Results. In the Results section, include the rationale or design of the experiments as well as the results; reserve extensive interpretation of the results for the Discussion section. Present the results as concisely as possible in one of the following: text, table(s), or figure(s). Avoid extensive use of graphs to present data that might be more concisely or more quantitatively presented in the text or tables. Limit photographs (particularly photomicrographs and electron micrographs) to those that are absolutely necessary to show the experimental findings. Number figures and tables in the order in which they are cited in the text, and be sure that all figures and tables are cited.

Discussion. The Discussion should provide an interpretation of the results in relation to previously published work and to the experimental system at hand and should not contain extensive repetition of the Results section or reiteration of the introduction. In short papers, the Results and Discussion sections may be combined.

Acknowledgments. The source of any financial support received for the work being published must be indicated in the Acknowledgments section. (It will be assumed that the absence of such an acknowledgment is a statement by the authors that no support was received.) The usual format is as follows: "This work was supported by Public Health Service grant CA-01234 from the National Cancer Institute."

Recognition of personal assistance should be given as a separate paragraph, as should any statements disclaiming endorsement or approval of the views reflected in the paper or of a product mentioned therein.

Appendixes. Appendixes that contain additional material to aid the reader are permitted. Titles, authors, and reference sections that are distinct from those of the primary article are not allowed. If it is not feasible to list the author(s) of the appendix in the byline or the Acknowledgments section of the primary article, rewrite the appendix so that it can be considered for publication as an independent article, either full-length or short-form style. Equations, tables, and figures should be labeled with the letter "A" preceding the numeral to distinguish them from those cited in the main body of the text.

References. (i) **References listed in the References section.** The References section must include all journal articles (both print and online), books and book chapters (both print and online), patents, theses and dissertations, published conference proceedings, meeting abstracts from published abstract books or journal supplements, letters (to the editor), and company publications, as well as in-press journal articles, book chapters, and books (publication title must be given). As we use the citation-name reference style, arrange the citations in **alphabetical order** (letter by letter, ignoring spaces and punctuation) by first-author surname and **number consecutively**. Provide the names of all the authors for each reference. All listed references must be cited parenthetically by number in the text. Since title and byline information that is downloaded from PubMed does not always show accents, italics, or special characters, authors should refer to the PDF files or hard-copy versions of the articles and incorporate the necessary corrections in the submitted manuscript. Abbreviate journal names according to the PubMed Journals Database (National Library of Medicine, National Institutes of Health; available at <http://www.ncbi.nlm.nih.gov/sites/entrez?db=journals>), the primary source for ASM style, but use periods on abbreviated words.

Follow the styles shown in the examples below for print references.

1. Alexander, T. W., et al. 2008. Effect of subtherapeutic administration of antibiotics on the prevalence of antibiotic-resistant *Escherichia coli* bacteria in feedlot cattle. *Appl. Environ. Microbiol.* 74:4405–4416.
2. Cox, C. S., B. R. Brown, and J. C. Smith. J. Gen. Genet., in press.* {Article title is optional; journal title is mandatory.}
3. da Costa, M. S., M. F. Nobre, and F. A. Rainey. 2001. Genus I. *Thermus* Brock and Freeze 1969, 295,^{AL} emend. Nobre, Trüper and da Costa 1996b, 605, p. 404–414. In D. R. Boone, R. W. Castenholz, and G. M. Garrity (ed.), *Bergey's manual of systematic bacteriology*, 2nd ed., vol. 1. Springer, New York, NY.
4. Elder, B. L., and S. E. Sharp. 2003. Cumitech 39, Competency assessment in the clinical laboratory. Coordinating ed., S. E. Sharp. ASM Press, Washington, DC.
5. Falagas, M. E., and S. K. Kasiakou. 2006. Use of international units when dosing colistin will help decrease confusion related to various formulations of the drug around the world. *Antimicrob. Agents Chemother.* 50:2274–2275. (Letter.) {"Letter" or "Letter to the editor" is allowed but not required at the end of such an entry.}
6. Fitzgerald, G., and D. Shaw. In A. E. Waters (ed.), *Clinical microbiology*, in press. EFH Publishing Co., Boston, MA.* {Chapter title is optional.}
7. Forman, M. S., and A. Valsamakis. 2003. Specimen collection, transport, and processing: virology, p. 1227–1241. In P. R. Murray, E. J. Baron, M. A. Pfaller, J. H. Tenover, and R. H. Tenover (ed.), *Manual of clinical microbiology*, 8th ed. ASM Press, Washington, DC.
8. Garcia, C. O., et al. 1996. Detection of salmonella DNA in synovial membrane and synovial fluid from Latin American patients. *Arthritis Rheum.* 39(Suppl.): S185. {Meeting abstract published in journal supplement.}
9. Green, P. N., D. Hood, and C. S. Dow. 1984. Taxonomic status of some methylotrophic bacteria, p. 251–254. In R. L. Crawford and R. S. Hanson (ed.), *Microbial growth on C₁ compounds*. Proceedings of the 4th International Symposium. American Society for Microbiology, Washington, DC.
10. Odell, J. C. April 1970. Process for batch culturing. U.S. patent 484,363,770. {Include the name of the patented item/process if possible; the patent number is mandatory.}
11. O'Malley, D. R. 1998. Ph.D. thesis. University of California, Los Angeles, CA. {Title is optional.}
12. Rotimi, V. O., N. O. Salako, E. M. Mohaddas, and L. P. Philip. 2005. Abstr. 45th Intersci. Conf. Antimicrob. Agents Chemother., abstr. D-1658. {Abstract title is optional.}
13. Smith, D., C. Johnson, M. Maier, and J. J. Maurer. 2005. Distribution of fimbrial, phage and plasmid associated virulence genes among poultry *Salmonella enterica* serovars, abstr. P-038, p. 445. Abstr. 105th Gen. Meet. Am. Soc. Microbiol. American Society for Microbiology, Washington, DC. {Abstract title is optional.}
14. Stratagene. 2006. Yeast DNA isolation system: instruction manual. Stratagene, La Jolla, CA. {Use the company name as the author if none is provided for a company publication.}

*A reference to an in-press ASM publication should state the control number (e.g., AAC00577-11) if it is a journal article or the name of the publication if it is a book.

Online references must provide essentially the same information that print references do. For online journal articles, posting or revision dates may replace the year of publication, and a DOI or URL may be provided in addition to or in lieu of volume and page numbers. Some examples follow.

1. Charlier, D., and N. Glansdorff. September 2004, posting date. Chapter 3.6.1.10, Biosynthesis of arginine and polyamines. In R. Curtiss III et al. (ed.), *EcoSal—Escherichia coli and Salmonella: cellular and molecular biology*. ASM Press, Washington, DC. <http://www.ecosal.org/>. {Note that each chapter has its own posting date.}
2. Dionne, M. S., and D. S. Schneider. 2002. Screening the fruitfly immune system. *Genome Biol.* 3: REVIEWS1010. <http://genomebiology.com/2002/3/4/reviews/1010>.
3. Smith, F. X., H. J. Merianos, A. T. Brunger, and D. M. Engelman. 2001. Polar residues drive association of polyleucine transmembrane helices. *Proc. Natl. Acad. Sci. U. S. A.* 98:2250–2255. doi:10.1073/pnas.041593698.
4. Winnick, S., D. O. Lucas, A. L. Hartman, and D. Toll. 2005. How do you improve compliance? *Pediatrics* 115:e718–e724.

Note: a posting or accession date is required for any online reference that is periodically updated or changed.

(ii) References cited in the text. References to unpublished data, manuscripts submitted for publication, unpublished conference presentations (e.g., a report or poster that has not appeared in published conference proceedings), personal communications, patent applications and patents pending, computer software, databases, and websites should be made parenthetically in the text as follows.

- ... similar results (R. B. Layton and C. C. Weathers, unpublished data).
- ... system was used (J. L. McInerney, A. F. Holden, and P. N. Brighton, submitted for publication).
- ... as described previously (M. G. Gordon and F. L. Rattner, presented at the Fourth Symposium on Food Microbiology, Overton, IL, 13 to 15 June 1989). {For nonpublished abstracts and posters, etc.}
- ... this new process (V. R. Smoll, 20 June 1999, Australian Patent Office). {For non-U.S. patent applications, give the date of publication of the application.}
- ... available in the GenBank database (<http://www.ncbi.nlm.nih.gov/genbank/index.html>).
- ... using ABC software (version 2.2; Department

of Microbiology, State University [<http://www.state.micro.edu/>]).

URLs for companies that produce any of the products mentioned in your study or for products being sold may not be included in the article. However, company URLs that permit access to scientific data related to the study or to shareware used in the study are permitted.

(iii) References related to supplemental material. References that are related only to supplemental material hosted by ASM or posted on a personal/institutional website should not be listed in the References section of an article; include them with the supplemental material itself.

(iv) Referencing ASM Accepts (publish-ahead-of-print) manuscripts. Citations of ASM Accepts manuscripts should look like the following example.

Wang, G. G., M. P. Pasillas, and M. P. Kamps. 15 May 2006. Persistent transactivation by Meis1 replaces Hox function in myeloid leukemogenesis models: evidence for co-occupancy of Meis1-Pbx and Hox-Pbx complexes on promoters of leukemia-associated genes. *Mol. Cell. Biol.* doi:10.1128/MCB.00586-06.

Other journals may use different styles for their publish-ahead-of-print manuscripts, but citation entries must include the following information: author name(s), posting date, title, journal title, and volume and page numbers and/or DOI. The following is an example:

Zhou, F. X., H. J. Merianos, A. T. Brunger, and D. M. Engelman. 13 February 2001, posting date. Polar residues drive association of polyleucine transmembrane helices. *Proc. Natl. Acad. Sci. U. S. A.* doi:10.1073/pnas.041593698.

Short-Form Papers

The short-form format is intended for the presentation of brief observations that do not warrant full-length papers. Submit short-form papers in the same way as full-length papers. They receive the same review, they are not published more rapidly than full-length papers, and they are not considered preliminary communications.

The title, running title (not to exceed 54 characters and spaces), byline, and correspondent footnote should be prepared as for a full-length paper. Each short-form paper must have an abstract of no more than 75 words. Do not use section headings in the body of the paper; combine methods, results, and discussion in a single section. Paragraph lead-ins are permissible. The text should be kept to a minimum and if possible should not exceed 1,000 words; the number of figures and tables should also be kept to a minimum. Materials and methods should be described in the text, not in figure legends

or table footnotes. Present acknowledgments as in full-length papers, but do not use a heading. The References section is identical to that of full-length papers.

Minireviews

Minireviews are brief (**limit of six printed pages exclusive of references**) biographical profiles, historical perspectives, or summaries of developments in fast-moving areas of chemotherapy. They must be based on published articles; they are not outlets for unpublished data. They may address any subject within the scope of AAC. For example, subject matter may range from structure-activity correlates among a group of semisynthetic cephalosporins to the comparative efficacies of new and old drugs in the prevention or treatment of diseases of microbial origin in humans.

Minireviews may be either solicited or proffered by authors responding to a recognized need. Irrespective of origin, Minireviews are subject to review and should be submitted via the online manuscript submission and peer review system. The cover letter should state whether the article was solicited and by whom.

Minireviews must have abstracts. Limit the abstract to 250 words or fewer. The body of the Minireview may have section headings and/or paragraph lead-ins.

Author biographies. At the editor's invitation, corresponding authors of Minireviews may submit brief biographical sketches (limit, 150 words) of each contributing author, to be published at the end of the article. If the editor asks you to submit a modified manuscript, you should submit biographical text and photos with your modification.

Commentaries

Commentaries are invited communications concerning topics relevant to the readership of AAC and are intended to engender discussion. Reviews of the literature, methods and other how-to papers, and responses targeted at a specific published paper are not appropriate. Commentaries are subject to review.

The length may not exceed four printed pages, and the format is like that of a Minireview (see above) except that Commentaries do not have abstracts. (In the submission form, use "NA" or "Not Applicable" in the space provided for the abstract.)

Letters to the Editor

Two types of Letters to the Editor may be submitted. The first type (Comment Letter) is intended for comments on final, typeset articles published in the journal (not on publish-ahead-of-print manuscripts) and must cite published references to support the writer's argument. The second type (New-Data Letter) may report new, concise findings that are not appropriate for publication as full-length or short-form papers.

Letters may be **no more than 500 words long and must be typed double-spaced**. Refer to a recently published Letter for correct formatting. Note that authors and affiliations are listed at the foot of the Letter. Provide only the primary affiliation for each author.

All Letters to the Editor must be submitted electronically, and the type of Letter (New Data or Comment) must be selected from the drop-down list in the submission form. For Letters commenting on published articles, the cover letter should state the volume and issue in which the article was published, the title of the article, and the last name of the first author. In the Abstract section of the submission form, put "Not Applicable." Letters to the Editor do not have abstracts. Both types of Letter must have a title, which must appear on the manuscript and on the submission form. Figures and tables should be kept to a minimum.

If the Letter is related to a published article, it will be sent to the editor who handled the article in question. If the editor believes that publication is warranted, he/she will solicit a reply from the corresponding author of the article and give approval for publication.

New-Data Letters will be assigned to an editor according to subject matter and will be reviewed by that editor and/or a reviewer.

Please note that some indexing/abstracting services do not include Letters to the Editor in their databases.

Errata

The Erratum section provides a means of correcting errors that occurred during the writing, typing, editing, or publication (e.g., a misspelling, a dropped word or line, or mislabeling in a figure) of a published article. Submit Errata via the online manuscript submission and peer review system (see "Submission, Review, and Publication Processes"). In the Abstract section of the submission form (a required field), put "Not Applicable." Upload the text of your Erratum as a Microsoft Word file. Please see a recent issue for correct formatting.

Authors' Corrections

The Author's Correction section provides a means of correcting errors of omission (e.g., author names or citations) and errors of a scientific nature that do not alter the overall basic results or conclusions of a published article (e.g., an incorrect unit of measurement or order of magnitude used throughout, contamination of one of numerous cultures, or misidentification of a mutant strain, causing erroneous data for only a [noncritical] portion of the study). Note that the addition of new data is not permitted.

For corrections of a scientific nature or issues involving authorship, including contributions and use or ownership of data and/or materials, all disputing parties must agree, in writing, to publication of the Correction. For omission of an author's name, letters must be signed by the authors of the article and the author whose name

was omitted. The editor who handled the article will be consulted if necessary.

Submit an Author's Correction via the online manuscript submission and peer review system (see "Submission, Review, and Publication Processes"). Select Erratum as the manuscript type; there is no separate selection for an Author's Correction, but your Correction will be published as such if appropriate. In the Abstract section of the submission form (a required field), put "Not Applicable." Upload the text of your Author's Correction as a Microsoft Word file. Please see a recent issue for correct formatting. Signed letters of agreement must be supplied as supplemental material (scanned PDF files).

Retractions

Retractions are reserved for major errors or breaches of ethics that, for example, may call into question the source of the data or the validity of the results and conclusions of an article. Submit Retractions via the online manuscript submission and peer review system (see "Submission, Review, and Publication Processes"). In the Abstract section of the submission form (a required field), put "Not Applicable." Upload the text of your Retraction as a Microsoft Word file. Letters of agreement signed by all of the authors must be supplied as supplemental material (scanned PDF files). The Retraction will be assigned to the editor in chief of the journal, and the editor who handled the paper and the chairperson of the ASM Publications Board will be consulted. If all parties agree to the publication and content of the Retraction, it will be sent to the Journals Department for publication.

ILLUSTRATIONS AND TABLES

Illustrations

Image manipulation. Computer-generated images may be processed only minimally. Processing (e.g., changing contrast, brightness, or color balance) is acceptable only if applied to all parts of the image, as well as to the controls, equally, and descriptions of all such adjustments and the tools used (both hardware and software) must be provided in the manuscript. Unprocessed data and files must be retained by the authors and be provided to the editor on request.

File types and formats. Illustrations may be continuous-tone images, line drawings, or composites. Color graphics may be submitted, but the cost of printing in color must be borne by the author. Suggestions about how to reduce costs and ensure accurate color reproduction are given below.

On initial submission, illustrations should be supplied as PDF files, with the legend on the same page, to assist review. At the modification stage, production quality digital files must be provided, along with text files for the

legends. The legends are copyedited and typeset for final publication, not included as part of the figure itself. All graphics submitted with modified manuscripts must be bitmap, grayscale, or in the RGB (preferred) or CMYK color mode. See "Color illustrations." Halftone images (those with various densities or shades) must be grayscale, not bitmap. AAC accepts TIFF or EPS files but discourages PowerPoint for either black-and-white or color images.

For instructions on creating acceptable EPS and TIFF files, refer to the Cadmus digital art website, <http://art.cadmus.com/da/index.jsp>. PowerPoint requires users to pay close attention to the fonts used in their images (see the section on fonts below). If instructions for fonts are not followed exactly, images prepared for publication are subject to missing characters, improperly converted characters, or shifting/obscuring of elements or text in the figure. For proper font use in PowerPoint images, refer to the Cadmus digital art website, http://art.cadmus.com/da/instructions/ppt_disclaimer.jsp.

We strongly recommend that before returning their modified manuscripts, authors check the acceptability of their digital images for production by running their files through Rapid Inspector, a tool provided at the following URL: <http://rapidinspector.cadmus.com/RapidInspector/zmw/index.jsp>. Rapid Inspector is an easy-to-use, Web-based application that identifies file characteristics that may render the image unusable for production.

If you require additional information, please send an e-mail inquiry to digitalart@cadmus.com.

Minimum resolution. It is extremely important that a high enough file resolution is used. All separate images that you import into a figure file must be at the correct resolution before they are placed. (For instance, placing a 72-dpi image in a 300-dpi EPS file will not result in the placed image meeting the minimum requirements for file resolution.) Note, however, that the higher the resolution, the larger the file and the longer the upload time. Publication quality will not be improved by using a resolution higher than the minimum. Minimum resolutions are as follows:

- 300 dpi for grayscale and color
- 600 dpi for combination art (lettering and images)
- 1,200 dpi for line art

Size. All graphics should be submitted at their intended publication size; that is, the image uploaded should be 100% of its print dimensions so that no reduction or enlargement is necessary. Resolution must be at the required level at the submitted size. Include only the significant portion of an illustration. White space must be cropped from the image, and excess space between panel labels and the image must be eliminated.

Maximum width for a 1-column figure: $3\frac{5}{16}$ inches (ca. 8.4 cm)

Maximum width for a 2-column figure: $6\frac{7}{8}$ inches (ca. 17.4 cm)

Minimum width for a 2-column figure: 4¼ inches (10.8 cm)

Maximum height: 9½ inches (23.0 cm)

Contrast. Illustrations must contain sufficient contrast to be viewed easily on a monitor or on the printed page.

Labeling and assembly. All final lettering and labeling must be incorporated into the figures. On initial submission, illustrations should be provided as PDF files, with the legend beneath each image, to assist review. At the modification stage, production quality digital figure files must be provided, along with text files for the legends. Put the figure number well outside the boundaries of the image itself. (Numbering may need to be changed at the copyediting stage.) Each figure must be uploaded as a separate file, and any multipanel figures must be assembled into one file; i.e., rather than uploading a separate file for each panel in a figure, assemble all panels in one piece and submit them as one file.

Fonts. To avoid font problems, set all type in one of the following fonts: Arial, Helvetica, Times Roman, European PI, Mathematical PI, or Symbol. Courier may be used but should be limited to nucleotide or amino acid sequences in which a nonproportional (monospace) font is required. All fonts other than these must be converted to paths (or outlines) in the application with which they were created.

Compression. When figure files are uploaded to the manuscript submission and peer review system, they may be compressed with WinZip.

Color illustrations. Color costs must be borne by the author. See "Publication Fees." **All figures submitted in color will be processed as color.** Adherence to the following guidelines will help to minimize costs and to ensure color reproduction that is as accurate as possible.

The online version is considered the version of record for AAC and all other ASM journals. To maximize online reproduction, color illustrations should be supplied in the RGB color mode, as either (i) RGB TIFF images with a resolution of at least 300 pixels per inch (raster files, consisting of pixels) or (ii) Illustrator-compatible EPS files with RGB color elements (vector files, consisting of lines, fonts, fills, and images). CMYK files are also accepted. Other than in color space, CMYK files must meet the same production criteria as RGB files. The RGB color space is the native color space of computer monitors and of most of the equipment and software used to capture scientific data, and it can display a wider range of colors (especially bright fluorescent hues) than the CMYK (cyan, magenta, yellow, black) color space used by print devices that put ink (or toner) on paper. For the print version (and reprints), ASM's print provider will automatically create CMYK versions of color illustrations from the supplied RGB versions. Color in the print journal may not match that in the online jour-

nal of record because of the smaller range of colors capable of being reproduced by CMYK inks on a printing press. For additional information on RGB versus CMYK color, refer to the Cadmus digital art site, http://art.cadmus.com/da/guidelines_rgb.jsp.

Drawings

Submit graphs, charts, complicated chemical or mathematical formulas, diagrams, and other drawings as finished products not requiring additional artwork or typesetting. All elements, including letters, numbers, and symbols, must be easily readable, and both axes of a graph must be labeled. Keep in mind that the journal is published both in print and online and that the same electronic files submitted by the authors are used to produce both.

When creating line art, please use the following guidelines:

(i) **All art must be submitted at its intended publication size.** For acceptable dimensions, see "Size," above.

(ii) **Avoid using screens (i.e., shading) in line art.** It can be difficult and time-consuming to reproduce these images without moiré patterns. Various pattern backgrounds are preferable to screens as long as the patterns are not imported from another application. If you must use images containing screens,

(a) Generate the image at line screens of 85 lines per inch or less.

(b) When applying multiple shades of gray, differentiate the gray levels by at least 20%.

(c) Never use levels of gray below 5% or above 95% as they are likely to fade out or become totally black when output.

(iii) Use thick, solid lines that are no finer than 1 point in thickness.

(iv) No type should be smaller than 6 points at the final publication size.

(v) Avoid layering type directly over shaded or textured areas.

(vi) Avoid the use of reversed type (white lettering on a black background).

(vii) Avoid heavy letters, which tend to close up, and unusual symbols, which the printer may not be able to reproduce in the legend.

(viii) If colors are used, avoid using similar shades of the same color and avoid very light colors.

In figure ordinate and abscissa scales (as well as table column headings), avoid the ambiguous use of numbers

with exponents. Usually, it is preferable to use the Système International d'Unités (SI) symbols (μ for 10^{-6} , m for 10^{-3} , k for 10^3 , and M for 10^6 , etc.). A complete listing of SI symbols can be found in the International Union of Pure and Applied Chemistry (IUPAC) publication *Quantities, Units and Symbols in Physical Chemistry* (RSC Publishing, Cambridge, United Kingdom, 2007); an abbreviated list is available at <http://old.iupac.org/reports/1993/homann/index.html>. Thus, a representation of 20,000 cpm on a figure ordinate should be made by the number 20 accompanied by the label kcpm.

When powers of 10 must be used, the journal requires that the exponent power be associated with the number shown. In representing 20,000 cells per ml, the numeral on the ordinate should be "2" and the label should be "10⁴ cells per ml" (not "cells per ml $\times 10^{-4}$ "). Likewise, an enzyme activity of 0.06 U/ml might be shown as 6 accompanied by the label "10⁻² U/ml." The preferred designation is 60 mU/ml (milliunits per milliliter).

Presentation of Nucleic Acid Sequences

Long nucleic acid sequences must be presented as figures in the following format to conserve space. Print the sequence in lines of approximately 100 to 120 nucleotides in a nonproportional (monospace) font that is easily legible when published with a line length of 6 inches (ca. 15.2 cm). If possible, lines of nucleic acid sequence should be further subdivided into blocks of 10 or 20 nucleotides by spaces within the sequence or by marks above it. Uppercase and lowercase letters may be used to designate the exon-intron structure or transcribed regions, etc., if the lowercase letters remain legible at a 6-inch (ca. 15.2-cm) line length. Number the sequence line by line; place numerals representing the first base of each line to the left of the lines. Minimize spacing between lines of sequence, leaving room only for annotation of the sequence. Annotation may include boldface, underlining, brackets, and boxes, etc. Encoded amino acid sequences may be presented, if necessary, immediately above or below the first nucleotide of each codon, by using the single-letter amino acid symbols. Comparisons of multiple nucleic acid sequences should conform as nearly as possible to the same format.

Figure Legends

On initial submission, to assist review, the legend should be incorporated in the image file and appear beneath the figure. At the modification stage, figure legends must be provided as text files separate from the image file.

Legends should provide enough information so that the figure is understandable without frequent reference to the text. However, detailed experimental methods must be described in the Materials and Methods section, not in a figure legend. A method that is unique to one of several experiments may be set forth in a legend only if the description is very brief (one or two sentences). Define all symbols used in the figure and define all abbreviations that are not used in the text.

TABLE 1. Distribution of protein and ATPase in fractions of dialyzed membranes^a

Membrane	Fraction	ATPase	
		U/mg of protein	Total U
Control	Depleted membrane	0.036	2.3
	Concentrated supernatant	0.134	4.82
E1 treated	Depleted membrane	0.034	1.98
	Concentrated supernatant	0.11	4.6

^a Specific activities of ATPase of nondepleted membranes from control and treated bacteria were 0.21 and 0.20, respectively.

Tables

Tables that contain artwork, chemical structures, or shading must be submitted as illustrations in an acceptable format at the modification stage. The preferred format for regular tables is Microsoft Word; however, WordPerfect and Acrobat PDF are also acceptable. Note that a straight Excel file is not currently an acceptable format. Excel files must be either embedded in a Word or WordPerfect document or converted to PDF before being uploaded. **If your modified manuscript contains PDF tables and is being submitted in Rapid Review, select "for reviewing purposes only" at the beginning of the file upload process.**

Tables should be formatted as follows. Arrange the data so that columns of like material read down, not across. The headings should be sufficiently clear so that the meaning of the data is understandable without reference to the text. See the "Abbreviations" section of these Instructions for those that should be used in tables. Explanatory footnotes are acceptable, but more-extensive table "legends" are not. Footnotes should not include detailed descriptions of the experiment. Tables must include enough information to warrant table format; those with fewer than six pieces of data will be incorporated into the text by the copy editor. Table 1 is an example of a well-constructed table.

Avoid tables (or figures) of raw data on drug susceptibility, therapeutic activity, or toxicity. Such data should be analyzed by an approved procedure, and the results should be presented in tabular form.

NOMENCLATURE

Chemical and Biochemical Nomenclature

The recognized authority for the names of chemical compounds is *Chemical Abstracts* (CAS; <http://www.cas.org/>) and its indexes. *The Merck Index*, 14th ed. (Merck & Co., Inc., Whitehouse Station, NJ, 2006), is also an excellent source. For guidelines to the use of biochemical terminology, consult *Biochemical Nomenclature and Related Documents* (Portland Press, London, United Kingdom, 1992), available at <http://www.chem.qmul.ac.uk/iupac/bibliog/white.html>, and the instructions to authors of the *Journal of Biological Chemistry* and the *Archives of Biochemistry and Biophysics* (first issues of each year).

Molecular weight should not be expressed in daltons; molecular weight is a unitless ratio. Molecular mass is expressed in daltons.

For enzymes, use the recommended (trivial) name as assigned by the Nomenclature Committee of the International Union of Biochemistry (IUB) as described in *Enzyme Nomenclature* (Academic Press, Inc., New York, NY, 1992) and its supplements and at <http://www.chem.qmul.ac.uk/iubmb/enzyme/>. If a nonrecommended name is used, place the proper (trivial) name in parentheses at first use in the abstract and text. Use the EC number when one has been assigned. Authors of papers describing enzymological studies should review the standards of the STRENDA Commission for information required for adequate description of experimental conditions and for reporting enzyme activity data (<http://www.beilstein-institut.de/en/projekte/strenda/guidelines/>).

Nomenclature of Microorganisms

Binary names, consisting of a generic name and a specific epithet (e.g., *Escherichia coli*), must be used for all microorganisms. Names of categories at or above the genus level may be used alone, but specific and subspecific epithets may not. A specific epithet must be preceded by a generic name, written out in full the first time it is used in a paper. Thereafter, the generic name should be abbreviated to the initial capital letter (e.g., *E. coli*), provided there can be no confusion with other genera used in the paper. Names of all taxa (kingdoms, phyla, classes, orders, families, genera, species, and subspecies) are printed in italics and should be italicized in the manuscript; strain designations and numbers are not. Vernacular (common) names should be in lowercase roman type (e.g., streptococcus, brucella). For *Salmonella*, genus, species, and subspecies names should be rendered in standard form: *Salmonella enterica* at first use, *S. enterica* thereafter; *Salmonella enterica* subsp. *arizonae* at first use, *S. enterica* subsp. *arizonae* thereafter. Names of serovars should be in roman type with the first letter capitalized: *Salmonella enterica* serovar Typhimurium. After the first use, the serovar may also be given without a species name: *Salmonella* Typhimurium, *S. Typhimurium*, or *Salmonella* serovar Typhimurium. For other information regarding serovar designations, see *Antigenic Formulae of the Salmonella Serovars*, 9th ed. (P. A. D. Grimont and F.-X. Weill, WHO Collaborating Centre for Reference and Research on *Salmonella*, Institut Pasteur, Paris, France, 2007; see <http://www.pasteur.fr/ip/portal/action/WebdriveActionEvent/oid/01s-000036-089>). For a summary of the current standards for *Salmonella* nomenclature and the Kaufmann-White criteria, see the article by Brenner et al. (*J. Clin. Microbiol.* 38:2465–2467, 2000), the opinion of the Judicial Commission of the International Committee on Systematics of Prokaryotes (*Int. J. Syst. Evol. Microbiol.* 55:519–520, 2005), and the article by Tindall et al. (*Int. J. Syst. Evol. Microbiol.* 55:521–524, 2005).

The spelling of bacterial names should follow the *Approved Lists of Bacterial Names (Amended) & Index of the*

Bacterial and Yeast Nomenclatural Changes (V. B. D. Skerman et al., ed., American Society for Microbiology, Washington, DC, 1989) and the validation lists and notification lists published in the *International Journal of Systematic and Evolutionary Microbiology* (formerly the *International Journal of Systematic Bacteriology*) since January 1989. In addition, two sites on the World Wide Web list current approved bacterial names: Bacterial Nomenclature Up-to-Date (http://www.dsmz.de/microorganisms/main.php?contentleft_id=14) and List of Prokaryotic Names with Standing in Nomenclature (<http://www.bacterio.cict.fr/>). If there is reason to use a name that does not have standing in nomenclature, the name should be enclosed in quotation marks in the title and at its first use in the abstract and the text and an appropriate statement concerning the nomenclatural status of the name should be made in the text. “*Candidatus*” species should always be set in quotation marks.

Since the classification of fungi is not complete, it is the responsibility of the author to determine the accepted binomial for a given organism. Sources for these names include *The Yeasts: a Taxonomic Study*, 5th ed. (C. P. Kurtzman, J. W. Fell, and T. Boekhout, ed., Elsevier Science, Amsterdam, Netherlands, 2010), and *Dictionary of the Fungi*, 10th ed. (P. M. Kirk, P. F. Cannon, and J. A. Stalpers, ed., CABI Publishing, Wallingford, Oxfordshire, United Kingdom, 2008); see also <http://www.speciesfungorum.org/Names/Fundic.asp>.

Names used for viruses should be those approved by the International Committee on Taxonomy of Viruses (ICTV) and reported on the ICTV Virus Taxonomy website (<http://www.ictvonline.org/index.asp>). In addition, the recommendations of the ICTV regarding the use of species names should generally be followed: when the entire species is discussed as a taxonomic entity, the species name, as with other taxa, is italic and has the first letter and any proper nouns capitalized (e.g., *Tobacco mosaic virus*, *Murray Valley encephalitis virus*). When the behavior or manipulation of individual viruses is discussed, the vernacular (e.g., tobacco mosaic virus, Murray Valley encephalitis virus) should be used. If desired, synonyms may be added parenthetically when the name is first mentioned. Approved generic (or group) and family names may also be used.

Microorganisms, viruses, and plasmids should be given designations consisting of letters and serial numbers. It is generally advisable to include a worker's initials or a descriptive symbol of locale or laboratory, etc., in the designation. Each new strain, mutant, isolate, or derivative should be given a new (serial) designation. This designation should be distinct from those of the genotype and phenotype, and genotypic and phenotypic symbols should not be included. Plasmids are named with a lowercase “p” followed by the designation in uppercase letters and numbers. To avoid the use of the same designation as that of a widely used strain or plasmid, check the designation against a publication database such as Medline.

Genetic Nomenclature

To facilitate accurate communication, it is important that standard genetic nomenclature be used whenever possible and that deviations or proposals for new naming systems be endorsed by an appropriate authoritative body. Review and/or publication of submitted manuscripts that contain new or nonstandard nomenclature may be delayed by the editor or the Journals Department so that they may be reviewed by the Genetics and Genomics Committee of the ASM Publications Board.

Before submission of manuscripts, authors may direct questions on genetic nomenclature to the committee's chairperson: Maria Costanzo (maria@genome.stanford.edu). Such a consultation should be mentioned in the manuscript submission letter.

Bacteria. The genetic properties of bacteria are described in terms of phenotypes and genotypes. The phenotype describes the observable properties of an organism. The genotype refers to the genetic constitution of an organism, usually in reference to some standard wild type. The guidelines that follow are based on the recommendations of Demerec et al. (Genetics 54:61–76, 1966).

(i) Phenotype designations must be used when mutant loci have not been identified or mapped. They can also be used to identify the protein product of a gene, e.g., the OmpA protein. Phenotype designations generally consist of three-letter symbols; these are not italicized, and the first letter of the symbol is capitalized. It is preferable to use Roman or Arabic numerals (instead of letters) to identify a series of related phenotypes. Thus, a series of nucleic acid polymerase mutants might be designated Pol1, Pol2, and Pol3, etc. Wild-type characteristics can be designated with a superscript plus (Pol⁺), and, when necessary for clarity, negative superscripts (Pol[−]) can be used to designate mutant characteristics. Lowercase superscript letters may be used to further delineate phenotypes (e.g., Str^r for streptomycin resistance). Phenotype designations should be defined.

(ii) Genotype designations are also indicated by three-letter locus symbols. In contrast to phenotype designations, these are lowercase italic (e.g., *ara his rps*). If several loci govern related functions, these are distinguished by italicized capital letters following the locus symbol (e.g., *araA araB araC*). Promoter, terminator, and operator sites should be indicated as described by Bachmann and Low (Microbiol. Rev. 44:1–56, 1980): e.g., *lacZp*, *lacAt*, and *lacZo*.

(iii) Wild-type alleles are indicated with a superscript plus (*ara⁺ his⁺*). A superscript minus is not used to indicate a mutant locus; thus, one refers to an *ara* mutant rather than an *ara[−]* strain.

(iv) Mutation sites are designated by placing serial isolation numbers (allele numbers) after the locus symbol (e.g., *araA1 araA2*). If only a single such locus exists or if it is not known in which of several related loci the mutation has occurred, a hyphen is used instead of the capital letter (e.g., *ara-23*). It is essential in papers reporting the isolation of new mutants that allele numbers

be given to the mutations. For *Escherichia coli*, there is a registry of such numbers: *E. coli* Genetic Stock Center (<http://cgsc.biology.yale.edu/>). For the genus *Salmonella*, the registry is *Salmonella* Genetic Stock Center (<http://people.ucalgary.ca/~kesander/>). For the genus *Bacillus*, the registry is *Bacillus* Genetic Stock Center (<http://www.bgsc.org/>).

(v) The use of superscripts with genotypes (other than + to indicate wild-type alleles) should be avoided. Designations indicating amber mutations (Am), temperature-sensitive mutations (Ts), constitutive mutations (Con), cold-sensitive mutations (Cs), production of a hybrid protein (Hyb), and other important phenotypic properties should follow the allele number [e.g., *araA230*(Am) *hisD21*(Ts)]. All other such designations of phenotype must be defined at the first occurrence. If superscripts must be used, they must be approved by the editor and defined at the first occurrence in the text.

Subscripts may be used in two situations. Subscripts may be used to distinguish between genes (having the same name) from different organisms or strains; e.g., *his_{E. coli}* or *his_{K-12}* for the *his* gene of *E. coli* or strain K-12, respectively, may be used to distinguish this gene from the *his* gene in another species or strain. An abbreviation may also be used if it is explained. Similarly, a subscript is also used to distinguish between genetic elements that have the same name. For example, the promoters of the *gln* operon can be designated *glnAp₁* and *glnAp₂*. This form departs slightly from that recommended by Bachmann and Low (e.g., *desC1p*).

(vi) Deletions are indicated by the symbol Δ placed before the deleted gene or region, e.g., Δ*trpA432*, Δ(*aroP-aceE*)419, or Δ(*hisQ-hisJ*)1256. Similarly, other symbols can be used (with appropriate definition). Thus, a fusion of the *ara* and *lac* operons can be shown as Φ(*ara-lac*)95. Likewise, Φ(*araB⁺-lacZ⁺*)96 indicates that the fusion results in a truncated *araB* gene fused to an intact *lacZ* gene, and Φ(*malE-lacZ*)97(Hyb) shows that a hybrid protein is synthesized. An inversion is shown as IN(*rmD-rmE*)1. An insertion of an *E. coli his* gene into plasmid pSC101 at zero kilobases (0 kb) is shown as pSC101 Ω(0kb::K-12*hisB*)4. An alternative designation of an insertion can be used in simple cases, e.g., *galT236::Tn5*. The number 236 refers to the locus of the insertion, and if the strain carries an additional *gal* mutation, it is listed separately. Additional examples, which utilize a slightly different format, can be found in the papers by Campbell et al. and Novick et al. cited below. It is important in reporting the construction of strains in which a mobile element was inserted and subsequently deleted that this fact be noted in the strain table. This can be done by listing the genotype of the strain used as an intermediate in a table footnote or by making a direct or parenthetical remark in the genotype, e.g., (F[−]), Δ*Mu* cts, or *mal::ΔMu* cts::*lac*. In setting parenthetical remarks within the genotype or dividing the genotype into constituent elements, parentheses and brackets are used without special meaning; brackets are used outside parentheses. To indicate the presence of an episome, pa-

rentheses (or brackets) are used (λ , F^+). Reference to an integrated episome is indicated as described above for inserted elements, and an exogenote is shown as, for example, W3110/ $F'8(gal^+)$.

For information about genetic maps of locus symbols in current use, consult Berlyn (Microbiol. Mol. Biol. Rev. 62:814–984, 1998) for *E. coli* K-12, Sanderson and Roth (Microbiol. Rev. 52:485–532, 1988) for *Salmonella* serovar Typhimurium, Holloway et al. (Microbiol. Rev. 43:73–102, 1979) for the genus *Pseudomonas*, Piggot and Hoch (Microbiol. Rev. 49:158–179, 1985) for *Bacillus subtilis*, Perkins et al. (Microbiol. Rev. 46:426–570, 1982) for *Neurospora crassa*, and Mortimer and Schild (Microbiol. Rev. 49:181–213, 1985) for *Saccharomyces cerevisiae*. For yeasts, *Chlamydomonas* spp., and several fungal species, symbols such as those given in the *Handbook of Microbiology*, 2nd ed. (A. I. Laskin and H. A. Lechevalier, ed., CRC Press, Inc., Cleveland, OH, 1988) should be used.

Conventions for naming genes. It is recommended that (entirely) new genes be given names that are mnemonics of their function, avoiding names that are already assigned and earlier or alternative gene names, irrespective of the bacterium for which such assignments have been made. Similarly, it is recommended that, whenever possible, orthologous genes present in different organisms receive the same name. When homology is not apparent or the function of a new gene has not been established, a provisional name may be given by one of the following methods. (i) The gene may be named on the basis of its map location in the style *yaaA*, analogous to the style used for recording transposon insertions (*zef*) as discussed below. A list of such names in use for *E. coli* has been published by Rudd (Microbiol. Mol. Biol. Rev. 62:985–1019, 1998). (ii) A provisional name may be given in the style described by Demerec et al. (e.g., *usg*, gene upstream of *folC*). Such names should be unique, and names such as *orf* or *genX* should not be used. For reference, the *E. coli* Genetic Stock Center's database includes an updated listing of *E. coli* gene names and gene products. It is accessible on the Internet (<http://cgsc.biology.yale.edu/index.php>). A list can also be found in the work of Riley (Microbiol. Rev. 57:862–952, 1993). For the genes of other bacteria, consult the references given above.

For prokaryotes, gene names should not begin with prefixes indicating the genus and species from which the gene is derived. (However, subscripts may be used where necessary to distinguish between genes from different organisms or strains as described in section v of "Bacteria" above.) For eukaryotes, such prefixes may be used for clarity when discussing genes with the same name from two different organisms (e.g., *ScURA3* versus *CaURA3*); the prefixes are not considered part of the gene name proper and are not italicized.

Locus tags. Locus tags are systematic, unique identifiers that are assigned to each gene in GenBank. All genes

mentioned in a manuscript should be traceable to their sequences by the reader, and locus tags may be used for this purpose in manuscripts to identify uncharacterized genes. In addition, authors should check GenBank to make sure that they are using the correct, up-to-date format for locus tags (e.g., uppercase versus lowercase letters and the presence or absence of an underscore, etc.). Locus tag formats vary between different organisms and also may be updated for a given organism, so it is important to check GenBank at the time of manuscript preparation.

"Mutant" versus "mutation." Keep in mind the distinction between a mutation (an alteration of the primary sequence of the genetic material) and a mutant (a strain carrying one or more mutations). One may speak about the mapping of a mutation, but one cannot map a mutant. Likewise, a mutant has no genetic locus, only a phenotype.

"Homology" versus "similarity." For use of terms that describe relationships between genes, consult the articles by Theissen (Nature 415:741, 2002) and Fitch (Trends Genet. 16:227–231, 2000). "Homology" implies a relationship between genes that have a common evolutionary origin; partial homology is not recognized. When sequence comparisons are discussed, it is more appropriate to use the term "percent sequence similarity" or "percent sequence identity," as appropriate.

Strain designations. Do not use a genotype as a name (e.g., "... subsequent use of *leuC6* for transduction ..."). If a strain designation has not been chosen, select an appropriate word combination (e.g., "another strain containing the *leuC6* mutation").

Viruses. The genetic nomenclature for viruses differs from that for bacteria. In most instances, viruses have no phenotype, since they have no metabolism outside host cells. Therefore, distinctions between phenotype and genotype cannot be made. Superscripts are used to indicate hybrid genomes. Genetic symbols may be one, two, or three letters. For example, a mutant strain of λ might be designated λ *Aam11 int2 red114 cI857*; this strain carries mutations in genes *cI*, *int*, and *red* and an amber-suppressible (*am*) mutation in gene *A*. A strain designated λ *att⁴³⁴ imm²¹* would represent a hybrid of phage λ that carries the immunity region (*imm*) of phage 21 and the attachment (*att*) region of phage 434. Host DNA insertions into viruses should be delineated by square brackets, and the genetic symbols and designations for such inserted DNA should conform to those used for the host genome. Genetic symbols for phage λ can be found in reports by Szybalski and Szybalski (Gene 7:217–270, 1979) and Echols and Muri- aldo (Microbiol. Rev. 42:577–591, 1978).

Eukaryotes. FlyBase (<http://flybase.org/>) is the genetic nomenclature authority for *Drosophila melanogaster*. WormBase (<http://wormbase.org/>) is the genetic nomenclature authority for *Caenorhabditis elegans*. When naming

genes for *Aspergillus* species, the nomenclature guidelines posted at http://www.aspergillus.org.uk/indexhome.htm?secure/sequence_info/nomenclature.htm~main should be followed, and the *Aspergillus* Genome Database (<http://www.aspgd.org>) should be searched to ensure that any new name is not already in use. For information about the genetic nomenclature of other eukaryotes, see the Instructions to Authors for *Eukaryotic Cell* and *Molecular and Cellular Biology*.

Transposable elements, plasmids, and restriction enzymes. Nomenclature of transposable elements (insertion sequences, transposons, and phage Mu, etc.) should follow the recommendations of Campbell et al. (Gene 5:197–206, 1979), with the modifications given in section vi of “Bacteria,” above. The Internet site where insertion sequences of eubacteria and archaea are described and new sequences can be recorded is <http://www-is.biotoul.fr/is.html>.

The system of designating transposon insertions at sites where there are no known loci, e.g., *zef-123::Tn5*, has been described by Chumley et al. (Genetics 91:639–655, 1979). The nomenclature recommendations of Novick et al. (Bacteriol. Rev. 40:168–189, 1976) for plasmids and plasmid-specified activities, of Low (Bacteriol. Rev. 36:587–607, 1972) for F' factors, and of Roberts et al. (Nucleic Acids Res. 31:1805–1812, 2003) for restriction enzymes, DNA methyltransferases, homing endonucleases, and their genes should be used whenever possible. The nomenclature for recombinant DNA molecules, constructed *in vitro*, follows the nomenclature for insertions in general. DNA inserted into recombinant DNA molecules should be described by using the gene symbols and conventions for the organism from which the DNA was obtained.

Tetracycline resistance determinants. The nomenclature for tetracycline resistance determinants is based on the proposal of Levy et al. (Antimicrob. Agents Chemother. 43:1523–1524, 1999). The style for such determinants is, e.g., Tet B; the space helps distinguish the determinant designation from that for phenotypes and proteins (TetB). The above-referenced article also gives the correct format for genes, proteins, and determinants in this family.

ABBREVIATIONS AND CONVENTIONS

Verb Tense

ASM strongly recommends that for clarity you use the past tense to narrate particular events in the past, including the procedures, observations, and data of the study that you are reporting. Use the present tense for your own general conclusions, the conclusions of previous researchers, and generally accepted facts. Thus, most of the abstract, Materials and Methods, and Results will be in the past tense, and most of the introduction and some of the Discussion will be in the present tense.

Be aware that it may be necessary to vary the tense in

a single sentence. For example, it is correct to say “White (30) demonstrated that XYZ cells grow at pH 6.8,” “Figure 2 shows that ABC cells failed to grow at room temperature,” and “Air was removed from the chamber and the mice died, which proves that mice require air.” In reporting statistics and calculations, it is correct to say “The values for the ABC cells are statistically significant, indicating that the drug inhibited . . .”

For an in-depth discussion of tense in scientific writing, see p. 191–193 in *How To Write and Publish a Scientific Paper*, 6th ed.

Abbreviations

General. Abbreviations should be used as an aid to the reader, rather than as a convenience to the author, and therefore their use should be limited. Abbreviations other than those recommended by the IUPAC-IUB (*Biochemical Nomenclature and Related Documents*, 1992) should be used only when a case can be made for necessity, such as in tables and figures.

It is often possible to use pronouns or to paraphrase a long word after its first use (e.g., “the drug” or “the substrate”). Standard chemical symbols and trivial names or their symbols (folate, Ala, and Leu, etc.) may also be used.

Define each abbreviation and introduce it in parentheses the first time it is used; e.g., “cultures were grown in Eagle minimal essential medium (MEM).” Generally, eliminate abbreviations that are not used at least three times in the text (including tables and figure legends).

Not requiring introduction. In addition to abbreviations for Système International d'Unités (SI) units of measurement, other common units (e.g., bp, kb, and Da), and chemical symbols for the elements, the following should be used without definition in the title, abstract, text, figure legends, and tables: DNA (deoxyribonucleic acid); cDNA (complementary DNA); RNA (ribonucleic acid); cRNA (complementary RNA); RNase (ribonuclease); DNase (deoxyribonuclease); rRNA (ribosomal RNA); mRNA (messenger RNA); tRNA (transfer RNA); AMP, ADP, ATP, dAMP, ddATP, and GTP, etc. (for the respective 5' phosphates of adenosine and other nucleosides) (add 2', 3', or 5' when needed for contrast); ATPase and dGTPase, etc. (adenosine triphosphatase and deoxyguanosine triphosphatase, etc.); NAD (nicotinamide adenine dinucleotide); NAD⁺ (nicotinamide adenine dinucleotide, oxidized); NADH (nicotinamide adenine dinucleotide, reduced); NADP (nicotinamide adenine dinucleotide phosphate); NADPH (nicotinamide adenine dinucleotide phosphate, reduced); NADP⁺ (nicotinamide adenine dinucleotide phosphate, oxidized); poly(A) and poly(dT), etc. (polyadenylic acid and polydeoxythymidylic acid, etc.); oligo(dT), etc. (oligodeoxythymidylic acid, etc.); UV (ultraviolet); PFU (plaque-forming units); CFU (colony-forming units); MIC (minimal inhibitory concentration); Tris [tris(hydroxymethyl)aminomethane]; DEAE (diethylaminoethyl); EDTA (ethylenediaminetetraacetic acid); EGTA [ethylene glycol-

bis(β -aminoethyl ether)-*N,N,N',N'*-tetraacetic acid]; HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid); PCR (polymerase chain reaction); and AIDS (acquired immunodeficiency syndrome). Abbreviations for cell lines (e.g., HeLa) also need not be defined.

The following abbreviations should be used without definition in tables:

amt (amount)	SE (standard error)
approx (approximately)	SEM (standard error of the mean)
avg (average)	
concn (concentration)	sp act (specific activity)
diam (diameter)	sp gr (specific gravity)
expt (experiment)	temp (temperature)
exptl (experimental)	tr (trace)
ht (height)	vol (volume)
mo (month)	vs (versus)
mol wt (molecular weight)	wk (week)
no. (number)	wt (weight)
prepn (preparation)	yr (year)
SD (standard deviation)	

Drugs and pharmaceutical agents. Should an author decide to abbreviate the names of antimicrobial agents in a manuscript, the following standard abbreviations are strongly recommended.

(i) Antibacterial agents. Amikacin, AMK; amoxicillin, AMX; amoxicillin-clavulanic acid, AMC; ampicillin, AMP; ampicillin-sulbactam, SAM; azithromycin, AZM; azlocillin, AZL; aztreonam, ATM; carbenicillin, CAR; cefaclor, CEC; cefadroxil, CFR; cefamandole, FAM; cefazolin, CFZ; cefdinir, CDR; cefditoren, CDN; cefepime, FEP; cefetamet, FET; cefixime, CFM; cefmetazole, CMZ; cefonicid, CID; cefoperazone, CFP; cefotaxime, CTX; cefotetan, CTT; cefoxitin, FOX; cefpodoxime, CPD; cefprozil, CPR; ceftazidime, CAZ; cefibuten, CTB; ceftizoxime, ZOX; ceftriaxone, CRO; cefuroxime (axetil or sodium), CXM; cephalixin, LEX; cephalothin, CEF; cephalirin, HAP; cephradine, RAD; chloramphenicol, CHL; cinoxacin, CIN; ciprofloxacin, CIP; clarithromycin, CLR; clinafloxacin, CLX; clindamycin, CLI; colistin, CST; daptomycin, DAP; dicloxacillin, DCX; dirithromycin, DTM; doxycycline, DOX; enoxacin, ENX; erythromycin, ERY; fleroxacin, FLE; fosfomycin, FOF; gatifloxacin, GAT; gentamicin, GEN; grepafloxacin, GRX; imipenem, IPM; kanamycin, KAN; levofloxacin, LVX; linezolid, LZD; lomefloxacin, LOM; loracarbef, LOR; meropenem, MEM; methicillin, MET; mezlocillin, MEZ; minocycline, MIN; moxalactam, MOX; moxifloxacin, MXF; nafcillin, NAF; nalidixic acid, NAL; netilmicin, NET; nitrofurantoin, NIT; norfloxacin, NOR; ofloxacin, OFX; oxacillin, OXA; penicillin, PEN; piperacillin, PIP; piperacillin-tazobactam, TZP; polymyxin B, PMB; quinupristin-dalfopristin (Synercid), Q-D; rifabutin, RFB; rifampin, RIF; rifapentine, RFP; sparfloxacin, SPX; spectinomycin, SPT; streptomycin, STR; teicoplanin, TEC; telithromycin, TEL; tetracycline, TET; ticarcillin, TIC; ticarcillin-clavulanic acid, TIM; tigecycline, TGC; tobramycin, TOB;

trimethoprim, TMP; trimethoprim-sulfamethoxazole, SXT; trovafloxacin, TVA; and vancomycin, VAN.

(ii) β -Lactamase inhibitors. Clavulanic acid, CLA; sulbactam, SUL; and tazobactam, TZB.

(iii) Antifungal agents. Amphotericin B, AMB; clotrimazole, CLT; flucytosine, 5FC; fluconazole, FLC; itraconazole, ITC; ketoconazole, KTC; nystatin, NYT; terbinafine, TRB; and voriconazole, VRC.

(iv) Antiviral agents. Acyclovir, ACV; cidofovir, CDV; famciclovir, FCV; foscarnet, FOS; ganciclovir, GCV; penciclovir, PCV; valacyclovir, VCV; and zidovudine, AZT.

The use of "nonstandard" abbreviations to designate names of antibiotics and other pharmaceutical agents generally will not be accepted, because the use of different abbreviations for a single agent has often caused confusion. If, on occasion, a nonstandardized abbreviation for a drug or pharmaceutical substance is used, it will be accepted under the following conditions: (i) it must be defined at the first use in the text, (ii) it must be unambiguous in meaning, and (iii) it must contribute to ease of assimilation by readers.

Chemical or generic names of drugs should be used; the use of trade names is not permitted. Avoid the ambiguous term "generation" when classes of drugs are described. When code names or corporate proprietary numbers are to be used, either the chemical structure of the compound or a published literature reference illustrating the chemical structure, if known, must be provided at the first occurrence of the code name or number. For compounds not identified by generic nomenclature, all previous or concurrent identification numbers or appellations should be listed in the manuscript.

Pharmacodynamic terminology. Pharmacodynamic indices (PDIs) must be introduced at their first occurrence in the text and follow guidelines set forth by Mouton et al. (*J. Antimicrob. Chemother.* 55:601–607, 2005). In Materials and Methods, it should be clearly stated how the PDIs were derived. The most common indices used are the following: AUC/MIC ratio (the area under the concentration-time curve over 24 h in steady state divided by the MIC), AUIC (the area under the inhibitory curve; note that the AUC/MIC ratio is not equal to the AUIC), % T_{MIC} (the cumulative percentage of a 24-h period that the drug concentration exceeds the MIC under steady-state pharmacokinetic conditions), C_{max}/MIC ratio (the peak level divided by the MIC), PTA (probability of target attainment), and CFR (cumulative fraction of response). Clear distinction should be made between % T_{MIC} , which is expressed as a percentage of the dosing interval, and T_{MIC} , expressed in hours. It is strongly recommended that the prefix *f* be used with an index (e.g., *f*AUC) if the free, unbound fraction of the drug is meant.

β -Lactamases

Studies performed to characterize a β -lactamase or the interaction of a compound with a β -lactamase (i.e., as a substrate, inhibitor, or inducer) should follow the guidelines set forth by Bush and Sykes (Antimicrob. Agents Chemother. 30:6–10, 1986). Assays that measure the hydrolysis of β -lactam antibiotics must be appropriate for the substrate examined (e.g., iodometric methods are not appropriate quantitative assays for substrates whose products are unknown). Reproducibility of results must be shown. When referring to β -lactamases, please use the functional designations defined by Bush et al. (Antimicrob. Agents Chemother. 39:1211–1233, 1995). Alternatively, if the amino acid sequence for the enzyme is known, the β -lactamases may be described by molecular class as initiated by Ambler (Philos. Trans. R. Soc. Lond. B Biol. Sci. 289:321–331, 1980).

A database of defining amino acid alterations for many β -lactamases is maintained at the Internet address <http://www.lahey.org/studies/>. The managers of that site should be consulted about the name of a potentially novel β -lactamase sequence before a new designation or number is proposed for publication.

***In Vitro* Susceptibility Tests**

Tabulate results of determinations of minimal inhibitory and bactericidal concentrations according to the range of concentrations of each antimicrobial agent required to inhibit or kill the members of a species or of each group of microorganisms tested, as well as the corresponding concentrations required to inhibit 50 and 90% of the strains (MIC_{50} and MIC_{90} , respectively) and those required to kill 50 and 90% of the strains (MBC_{50} and MBC_{90} , respectively). The MIC_{50} and MIC_{90} reported should be the actual concentrations tested that inhibited 50 and 90%, respectively, of the strains. They should not be values calculated from the actual data obtained. When only six to nine isolates of a species are tested, tabulate only the MIC range of each antimicrobial agent tested.

If more than a single drug is studied, insert a column labeled "Test agent" between the columns listing the organisms and the columns containing the numerical data and record data for each agent in the same isolate order. Cumulative displays of MICs or MBCs in tables or figures are acceptable only under unusual circumstances.

The percentage of strains susceptible and/or resistant to an antibiotic at its breakpoint concentration may be given only if an appropriate breakpoint has been approved, as by the Clinical and Laboratory Standards Institute, 940 W. Valley Rd., Suite 1400, Wayne, PA 19087-1898. In the absence of approved breakpoints, authors cannot assign breakpoints or use breakpoints from related antibiotics. An exploratory analysis tabulating the percentage of strains inhibited over a range of concentrations is acceptable.

Bactericidal tests must be performed with a sufficient inoculum ($>5 \times 10^5$ CFU/ml) and subculture volume (0.01 ml) to ensure accurate determination of the 99.9% killing

endpoint, as described by Pearson et al. (Antimicrob. Agents Chemother. 18:699–708, 1980) and Taylor et al. (Antimicrob. Agents Chemother. 23:142–150, 1983). Inoculum size and subculture volume are also critical to studies of combinations of antimicrobial agents.

Synergy is defined in two-dimensional or checkerboard tests when the fractional inhibitory concentration (FIC) or fractional bactericidal concentration (FBC) index (Σ) is ≤ 0.5 . In killing curves, synergy is defined as a $\geq 2\text{-log}_{10}$ decrease in CFU per milliliter between the combination and its most active constituent after 24 h, and the number of surviving organisms in the presence of the combination must be $\geq 2\text{ log}_{10}$ CFU/ml below the starting inoculum. At least one of the drugs must be present in a concentration which does not affect the growth curve of the test organism when used alone. Antagonism is defined by a Σ FIC or Σ FBC of >4.0 .

When standard twofold-dilution schemes are used to determine checkerboard interactions, the inherent variability of the method casts doubt on the significance of interactions represented by Σ FICs or Σ FBCs of >0.5 but ≤ 4 . Therefore, such interactions, if labeled at all, should be termed "indifferent." Alternatively, indices in this range may be described as "nonsynergistic" or "nonantagonistic," as appropriate. The technically imprecise term "additive" should be avoided as it is too easily misunderstood. See reports by W. R. Greco et al. (Pharmacol. Rev. 47:331–385, 1995), F. C. Odds (J. Antimicrob. Chemother. 52:1, 2003), and M. D. Johnson et al. (Antimicrob. Agents Chemother. 48:693–715, 2004) for further discussion of these issues.

For killing curve tests, the minimal, accurately countable number of CFU per milliliter must be stated and the method used for determining this number must be described. In the absence of any drug and with a sample size of 1 ml, this number is 30 (1.5 in \log_{10}) CFU. If procedures for drug inactivation or removal have not been performed, the author must state how drug carryover effects were eliminated or quantified. For drugs showing an inoculum effect, mere dilution below the MIC obtained in standard tests is not sufficient.

Clinical Trials

(i) **Criteria for enrollment.** The methods used to find and enroll patients and the criteria for enrollment in a clinical trial should be stated. In addition, the time period (month/year to month/year) of the enrollment should be specified. It should be indicated, if appropriate, that written informed consent was obtained and that the trial was approved by the pertinent committee on human subjects.

(ii) **Method of randomization.** Randomized, double-blind studies are preferred. Comparisons using historical controls are usually regarded as questionable unless the differences in outcome between the groups are dramatic and almost certainly the result of the new intervention. The rationale for the choice of the control group should be explained. The sample size should be justified, and the method of randomization should be stated.

(iii) Criteria for determining whether a case is evaluable. The minimum criteria for evaluability should be stated explicitly. For example, it should be stated that the minimum criterion for evaluability was *a* or the combination of *b* and *c* rather than *a*, *b*, and *c* without designating which were the minimum criteria. The criteria for evaluability are usually different from those for enrollment.

(iv) Reasons for nonevaluability. State the number of patients in each group who were excluded from evaluation and the reason(s) for each exclusion.

(v) Criteria for assessment. Define each outcome for each category of assessment (e.g., "clinical outcomes were classified as cure, improvement, and failure; microbiological outcomes were classified as eradication, persistence, and relapse"). The frequency and timing of such assessments in relation to treatment should be stated. Specify any changes made in the study regimen(s) during the trial; the results for regimens with and without such modification generally should be stated separately. The criteria (questionnaires, results of specific laboratory tests) for evaluation of adverse effects should be stated, as should the period encompassed in the assessment and the time of assessment in relation to the time of treatment (e.g., daily during treatment). Some authors prefer to consider superinfections as failures of treatment, whereas others prefer to consider them separately or even as adverse effects. In any event, the manuscript should state the number of superinfections with each regimen and should differentiate between superinfections and colonization. The duration of follow-up should be mentioned.

(vi) Statistical analyses. The type of statistical test should be stated and, when appropriate, the reason for the choice of test should be given. References should be given for statistical procedures other than the *t* test, chi-square test, and Wilcoxon rank sum test. The comparability of the treatment groups at the baseline should be evaluated statistically.

For a review of some common errors associated with statistical analyses and reports, plus guidelines on how to avoid them, see the article by Olsen (Infect. Immun. 71:6689–6692, 2003).

For a review of basic statistical considerations for virology experiments, see the article by Richardson and Overbaugh (J. Virol. 79:669–676, 2005).

(vii) Beta error. For trials which show no statistically significant difference between regimens, the authors should calculate the probability (β) of a type II error and the power of the study ($1 - \beta$) to detect a specified clinically meaningful difference in efficacy between the regimens. For further details, see the article by Freiman et al. (N. Engl. J. Med. 299:690–694, 1978). Alternatively, or in addition, the authors should indicate the magnitude of difference between the regimens that

could have been detected at a statistically significant level with the number of evaluable patients studied.

For further details, see the editorial on guidelines for clinical trials (Antimicrob. Agents Chemother. 33:1829–1830, 1989).

Reporting Numerical Data

Standard metric units are used for reporting length, weight, and volume. For these units and for molarity, use the prefixes m, μ , n, and p for 10^{-3} , 10^{-6} , 10^{-9} , and 10^{-12} , respectively. Likewise, use the prefix k for 10^3 . Avoid compound prefixes such as m μ or $\mu\mu$. Use $\mu\text{g/ml}$ or $\mu\text{g/g}$ in place of the ambiguous ppm. Units of temperature are presented as follows: 37°C or 324 K.

When fractions are used to express units such as enzymatic activities, it is preferable to use whole units, such as g or min, in the denominator instead of fractional or multiple units, such as μg or 10 min. For example, "pmol/min" is preferable to "nmol/10 min," and " $\mu\text{mol/g}$ " is preferable to "nmol/ μg ." It is also preferable that an unambiguous form such as exponential notation be used; for example, " $\mu\text{mol g}^{-1} \text{min}^{-1}$ " is preferable to " $\mu\text{mol/g/min}$." Always report numerical data in the appropriate SI units.

Representation of data as accurate to more than two significant figures must be justified by presentation of appropriate statistical analyses.

For a review of some common errors associated with statistical analyses and reports, plus guidelines on how to avoid them, see the article by Olsen (Infect. Immun. 71:6689–6692, 2003).

For a review of basic statistical considerations for virology experiments, see the article by Richardson and Overbaugh (J. Virol. 79:669–676, 2005).

Isotopically Labeled Compounds

For simple molecules, labeling is indicated in the chemical formula (e.g., $^{14}\text{CO}_2$, $^3\text{H}_2\text{O}$, and $\text{H}_2^{35}\text{SO}_4$). Brackets are not used when the isotopic symbol is attached to the name of a compound that in its natural state does not contain the element (e.g., $^{32}\text{S-ATP}$) or to a word that is not a specific chemical name (e.g., ^{131}I -labeled protein, ^{14}C -amino acids, and ^3H -ligands).

For specific chemicals, the symbol for the isotope introduced is placed in square brackets directly preceding the part of the name that describes the labeled entity. Note that configuration symbols and modifiers precede the isotopic symbol. The following examples illustrate correct usage:

^{14}C urea	$[\gamma\text{-}^{32}\text{P}]\text{ATP}$
L-[methyl- ^{14}C]methionine	UDP-[U- ^{14}C]glucose
[2,3- ^3H]serine	<i>E. coli</i> [^{32}P]DNA
$[\alpha\text{-}^{14}\text{C}]\text{lysine}$	fructose 1,6-[1- ^{32}P]bisphosphate

AAC follows the same conventions for isotopic labeling as the *Journal of Biological Chemistry*, and more-detailed information can be found in the instructions to authors of that journal (first issue of each year).